

An Investigation into the Effects of Coldshock on the Eastern Tiger Swallowtail Butterfly *Pterourus (Papilio) glaucus* (C. Linnaeus 1758) (Lepidoptera: Papilionidae)

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Abstract - This paper examines and documents the dramatic effects achieved through coldshock experiments performed on the North American Swallowtail butterfly *Pterourus (Papilio) glaucus*. For this study, duplicate consecutive experiments were conducted on two different lineages of *P. glaucus*, the first from extreme southern Illinois, U.S.A., and the second from northern Illinois, U.S.A. Both lineages were initiated from wild-collected normal looking dark morph female individuals that had mated prior to capture. The southern lineage was bred for five generations and yielded over 500 individuals. The northern lineage was bred for three generations and yielded more than 1500 individuals. This paper summarizes and compares the results from both experiments and details the technique used, the various types of aberrations identified, and offers some hypotheses as to the underlying causes of several of the resulting aberrant wing patterns. In the first series of experiments, it was demonstrated that the coldshock technique caused a wing-wide incremental reversion of the female dark morph into females resembling the ancestral yellow morph through a virtually uninterrupted cline of dusted looking intermediate morphs over the course of five generations. The second series of experiments resulted in coldshock-induced aberrations in the dark morph females encompassing a completely different range of variation than previously encountered in the first experiment, including a wider variation in the extent and positioning of the affected pigment colors, more pronounced zonal pigment aberrations, a long series of specimens in which wing-wide insufficient scale quantities resulted in semi-transparent wings, and specimens in which a disproportionate lightening of the ventral surface occurred, creating several distinctive reproducible patterns. Most of these aberrations did not occur in the first experiment. In addition to these severe aberrations produced in the females, several minor pattern aberrations were identified in both males and females that are presumed to be either caused by or enhanced by the coldshock. This is the first paper detailing coldshock in *P. glaucus* that discusses the results of multiple generations of inbred coldshocked individuals, and the first account of the heritability of coldshock-initiated aberrant patterns in a succeeding generation of inbred non-coldshocked *P. glaucus* individuals. Also discussed is the importance of the stripe and border pattern elements, their relative stability and resistance to alteration by coldshock, and the role they play in the formation of various coldshock aberrations. The coldshock-induced effects of *Pattern Drift*, *Pattern Breakdown*, *Color Reversion*, and *Color Replacement* are introduced and discussed as they relate to *P. glaucus*. Finally, a discussion and limited degree of speculative analysis is offered for each of the identified wing color and pattern aberrations.

Keywords: Coldshock, Melanism, Pattern Drift, Pattern Breakdown, Pattern Stability, Aberrant Bracketing, Aberrant Trajectory, Aberrant Clustering, Pistol Grip Aberration, Bow Tie Aberration, Banded Aberration, Color Replacement, Color Reversion, *P. glaucus* ab. *magnificus* nov., Yellow Forewing Discal Cell Spot, Insufficient Scale Quantity, *P. glaucus* ab. *pellis* nov., Sigma Aberration, White Triangles Aberration, Homeotic Aberrations, Diffusion Barrier

INTRODUCTION

Perhaps no other butterfly species in North America is more spectacular and variable than *Pterourus (Papilio) glaucus*, the Eastern Tiger Swallowtail. It is bold and beautiful, common and widespread, and is a good subject for numerous avenues of study. Because its female is dimorphic, *P. glaucus* provides us with a unique opportunity to explore wing color and pattern development encompassing a wider range of options than is possible in a monomorphic species. *P. glaucus* has demonstrated throughout our experiments that it is capable of significantly greater variation in wing color patterns than previously assumed. In particular, the dark morph female can be induced through the use of coldshock to reveal innate

color capabilities that are not typically encountered in the wild. Once revealed, these abnormal color capabilities can be intensified by coldshock, and in conjunction with selective breeding and inbreeding, these aberrant color patterns can eventually attain a degree of heritability without further coldshock in subsequent generations.

P. glaucus is very unique among the Swallowtails. The male is bright yellow, with jet black borders and black "tiger" stripes running vertically down its forewings and hind wings. Its female is dimorphic, with its yellow morph being substantially similar to the male, giving rise to the hypothesis that the yellow female is the original ancestral form. In addition, closely related species like *P. rutulus* Lucas, 1852, *P.*

multicaudata W.F. Kirby, 1884, and *P. canadensis* Rothschild & Jordan, 1906, never developed dark morph females, which also lends credence to this hypothesis. At first glance, the dark morph female of *P. glaucus* appears to be a nearly all-black butterfly. However, it is much more unique than that. The dark morph is actually a melanic duplicate of the yellow morph, with all of its underlying stripe and border pattern elements being identical in both size and location. In the dark morph, the black pattern elements virtually blend in with the dark melanized background. No other Swallowtails possess this exact same underlying pattern relationship between their dimorphic forms. Remembering that the black pattern exists in the dark morph, even though seemingly hidden, will facilitate the reader's understanding of the coldshock-induced aberrations described in this paper.

Both morphs of the female of *P. glaucus* live sympatrically in more or less equal numbers throughout its range, and the males readily mate with either female color morph without appearing to have a mating selection preference. In our previous *P. glaucus* breeding experiments, by far the most interesting aberrations were obtained from the progeny of dark morph mothers. The dark morph *P. glaucus* is presumed to be a mutated morph that evolved to mimic the unpalatable *Battus philenor* C. Linnaeus, 1771, even existing in locations where *B. philenor* is not resident. Some have even called the yellow morph of *P. glaucus* the "wild type" and the dark morph the "mutant type" (Koch, et. al. 1998). This explanation of how the yellow morph gave rise to the dark morph is considered axiomatic, and may provide some insight into the underlying basis for the obtained coldshock results detailed in this paper.

The experiments examined in this paper were conducted in two stages. In the first stage, which will be called PCE1 (Perlman Coldshock Experiment 1) throughout this paper, coldshock was administered to the progeny and descendants of a single dark morph *P. glaucus* female from Alto Pass, IL, U.S.A. (far southern Illinois), (Fig. 1). In the second stage, which will be called PCE2 (Perlman Coldshock Experiment 2) throughout this paper, the same coldshock regimen was administered to the progeny and descendants of four dark morph *P. glaucus* females from Elgin, IL, U.S.A. (northern Illinois), (Fig. 1).

The original intent and purpose of this experiment was to investigate if, and in what manner, coldshock would affect the wing patterns and coloration in *P. glaucus*. Our goal was to produce as many aberrant specimens as possible and identify as many different types of aberrations as possible. In short, this was intended to be a qualitative rather than a quantitative experiment. Therefore, some of the conclusions and analysis presented later in this paper must be tempered with the qualification that bulk rearing techniques were used along with selective breeding and inbreeding. The aberrant specimens speak for themselves as to demonstrating what is possible through the use of coldshock, but there was no attempt made to quantify or determine the relative influences and contributions of coldshock, selective breeding, and inbreeding.

Our coldshock experiments produced a large number of aberrant wing patterns. Some aberrant patterns were common to both PCE1 and PCE2, and some were unique to either PCE1 or PCE2. As a result of the large number of different aberrations produced during the course of these experiments, we soon realized the efficacy of creating names that could be easily associated with the various aberrant forms. We realize that this practice might be frowned upon, but for us at least, the names vastly simplified our ability to recognize and discuss the various aberrations. As our list of names increased and evolved over the course of this experiment, we tried to keep most of the names simple and descriptive. However, in a few instances where several different aberrant patterns were present in the same specimen, we resorted to using names that were less descriptive. Many of our names were incorporated into the writing of this paper, and we hope that any criticism of their inclusion will be overshadowed by their usefulness.

MATERIALS AND METHODS

For the purposes of this series of experiments, *coldshock* is defined as an abrupt chilling of an early stage chrysalis, to a near-freezing temperature that will disrupt the biological processes that determine and synthesize the colors of wing scale pigments, while still allowing the chrysalis to develop in a modified way that will not trigger diapause.

Both of our experiments began with wild-collected dark morph females of *P. glaucus* that had been mated in the wild prior to our collecting them (Fig. 1). The butterflies were placed in cages with potted *Prunus serotina* plants. The ova were obtained on the plants and the larvae were allowed to reach 3rd instar on the potted plants. At 3rd instar, we transferred the larvae to individual containers and raised them on high quality *Prunus serotina* leaves until pupation. The indoor room temperature was not controlled during larval growth, but averaged 65° to 75° F (18° to 24° C), with a photo period averaging 14 to 16 hours per day.

Our coldshock technique consisted of monitoring the prepupal larvae closely, and noting the time of the final larval molt. We selected a time frame of 3 to 5 hours after the final molt to abruptly chill the new chrysalides to a temperature averaging between 35° and 38° F (2° to 4° C). We selected a minimum of 3 hours after final molt to ensure that each chrysalis successfully attained its intended shape with sufficient hardness to avoid handling damage. The chrysalides were kept refrigerated in separate containers for 72 hours, after which time they were abruptly returned to room temperature and allowed to develop normally. There is no indication that the chrysalides entered diapause, and they all eclosed within two weeks or less after being returned to room temperature. The chrysalides exhibited the full range of normal camouflage coloration that we observe in every generation. There appears to be no correlation between chrysalis coloration and any of the various coldshock-induced aberrant patterns in the adults.

Due to the number of generations bred during both the

PCE1 and PCE2 experiments, one generation in each of the experiments required an overwintering diapause. To induce the overwintering diapause, the newly formed chrysalides were kept at room temperature for 72 hours, after which time they were gradually chilled over a 24-hour period to a final temperature of approximately 28° to 35° F (-2° to 2° C). The chrysalides were overwintered at this relatively constant temperature for the duration of the winter. We removed the chrysalides weekly to spray them with water, but we did not allow the chrysalides to thaw. In the PCE1 experiment, the F1, F2, F4, and F5 generations were coldshocked as described. The PCE1 F3 generation was not coldshocked, but instead diapaused and overwintered as described. In the PCE2

experiment, half of the F1 generation was coldshocked as described. The remaining half of the PCE2 F1 generation was coldshocked, thawed, and then diapaused as described on the third day after the coldshock procedure was completed. The PCE2 F2 and F3 generations were coldshocked as described. (The PCE2 lineage was subsequently used as breeding stock for a follow-up experiment. Coldshock was discontinued after the F3 generation. However, aberrant specimens similar to those in PCE2 continued to appear in subsequent generations. Some of these aberrant specimens up to and including the F6 generation are included in this paper as further examples of coldshock-induced aberrations.)



Fig. 1 Comparison Of *P. glaucus* PCE1 Mother (Alto Pass, IL) To PCE2 Mothers (Elgin, IL)

The left four photos in this plate show various views of the single Alto Pass mother of the PCE1 experiment. The yellow Forewing Discal Cell Spots are obvious on both the dorsal and ventral surfaces, and this trait was inherited to varying degrees by most of its female descendents. The dorsal yellow dusting is most evident in the photo at the bottom right, especially on the forewings. In the top left photo, the yellow dusting is quite obvious on both the ventral forewings and hind wings, more easily revealing the underlying pattern stripes, and seeming to be more concentrated in the wing cells at the anal margins of both wings. Yellow dusting is also visible on the underside of the abdomen. Finally, the ventral forewings show a distinct yellow shading and lightening of the marginal border. The four photos at right (dorsal on top row, ventral on bottom row) represent typical examples of the types of coloration and pattern seen in our four PCE2 mothers. The darkness of the melanization is quite evident in the two specimens. The left specimen is almost totally black. The right specimen has lighter apices on the forewings, but they are NOT dusted with yellow.

RESULTS

(Author's Note: The Results section of this paper is much longer than in most papers due to the vast number of unique aberrations produced by our experiments. Our intention is to show examples of every coldshock-induced aberration in this section of the paper. For the coldshock aberrations that can be briefly explained, these explanations will accompany the photos in this section. For those aberrations that require more detailed explanations, brief explanations will accompany the photo plates with fuller coverage to be found in the Analysis section of this paper. In some cases, additional background information and additional photos, etc. will be found in the Supplementary Materials section of this paper.)

The coldshock-induced aberrations in the males consisted primarily of pattern aberrations, while the aberrations

produced in the females encompassed a very wide assortment of both pattern *and* color aberrations. Many of these wing color and pattern aberrations occurred immediately in the F1 generations of both the PCE1 and PCE2 experiments. The female F1 aberrations in both experiments were intentionally exploited as precursor aberrations that significantly intensified in the later generations due to selective inbreeding compounded by the additional application of coldshock. We refer to this process as Aberrant Bracketing. (See Supplementary Materials section for a fuller explanation of Aberrant Bracketing.)

In the PCE1 experiment, the most striking female aberration to occur was a wing-wide "yellowing" of the melanic background scales which reveals the underlying black stripe and border pattern. In the PCE2 experiment, very little yellowing occurred. Instead, the primary female aberrations

fell into three main categories: blue based aberrations, disproportionate lightening of the ventral surface, and insufficient scale quantity aberrations resulting in specimens developing semi-transparent wings. All of the female aberration types in PCE1 and PCE2 ranged from minor to extreme in their intensity.

All of the various male and female aberrations that we identified are presented through photographs and brief text in the following pages, beginning with the male aberrations, followed by the female aberrations. There are additional examples of some of the aberrations in the Supplementary Materials section. Many of the photo plates refer to specific

locations on the wings that can be located on the *P. glaucus* Wing Pattern and Venation Maps (Supplementary Materials Figs. 33 & 34).

Pattern Drift and Pattern Breakdown Aberrations in *P. glaucus* Males

The coldshock-induced aberrations in the males consisted almost exclusively of distortions in the black stripe and border pattern elements. We produced no male specimens that had pigment color aberrations. Both the PCE1 and PCE2 males exhibited similar types of pattern aberrations, with most of

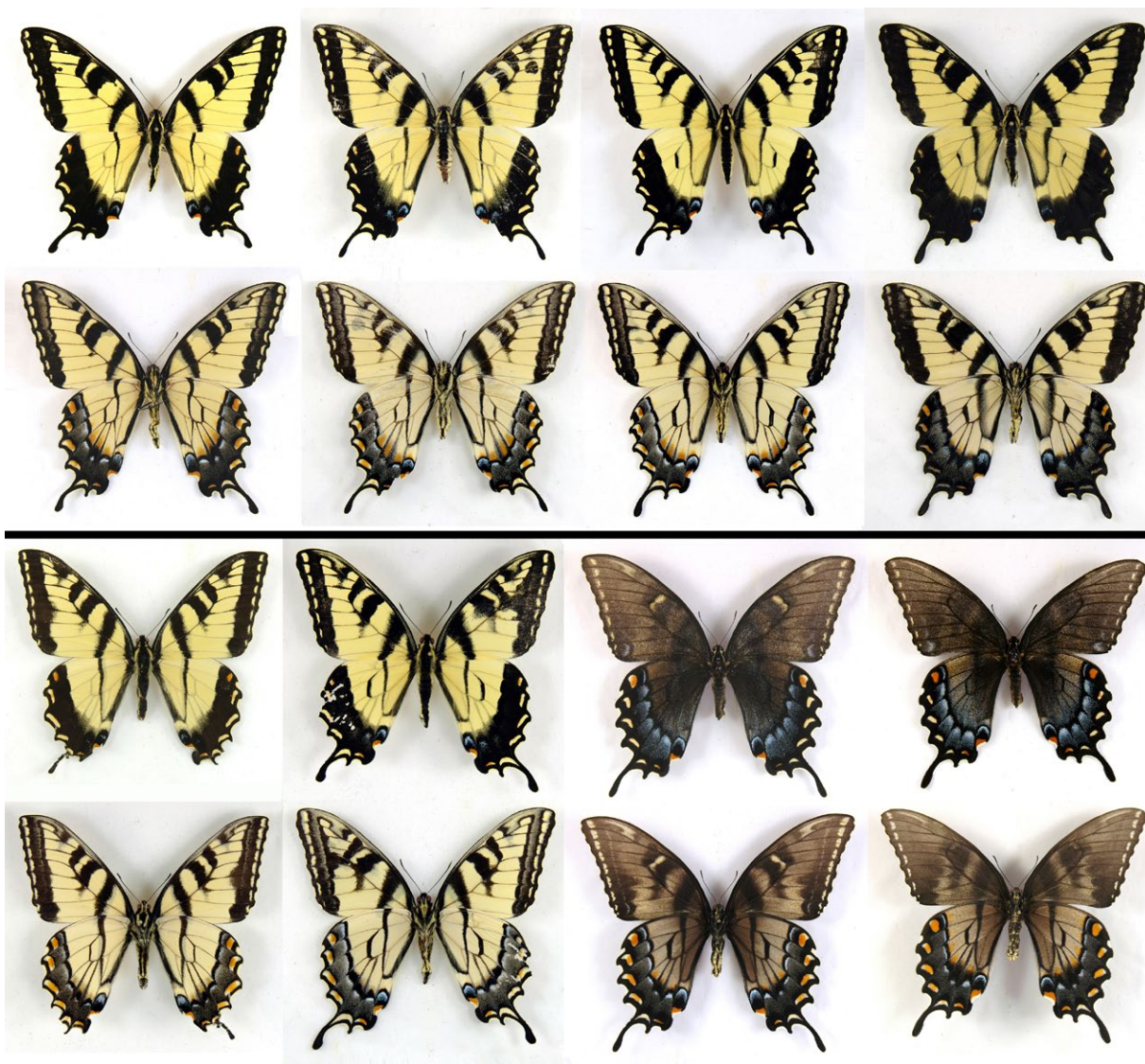


Fig. 2 Examples Of Pattern Drift Aberrations In Males And Females.

The sample specimens above are typical coldshock-induced Pattern Drift aberrations. The top 4 specimens (dorsal and ventral) exhibit a limited degree of stripe scalloping, but prominently display either stripe thickening and convergence of Forewing Stripes 2 and 3, or encroachment of the Forewing Border Proximal Band. Notice that there is a degree of correlation of these aberrations on the ventral surfaces as well. The bottom two male specimens are additional examples of border encroachment. The bottom two female specimens demonstrate forewing border encroachment which is minimally visible on the dorsal surface, but very pronounced on the ventral surface due to the disproportional lightening caused by the coldshock. Also, Forewing Stripe 4 is almost nonexistent in both specimens. Notice that the encroaching border is darker than the surrounding brown background scales, indicating that the encroaching border has a higher saturation level. The encroaching border has retained its original darker programming, even though the border has been displaced by Pattern Drift. Finally, notice the yellow Forewing Discal Cell Spots and Pistol Grip markings in the first female in the bottom row. These aberrations occurred in both PCE1 and PCE2.

the affected individuals varying in severity from minimally to moderately distorted. A few specimens displayed extremely disrupted patterns.

The most typical distortions in the stripe pattern include stripe dislocation, stripe extension or shortening, stripe scalloping, stripe diffusion or fuzziness, and stripe widening or narrowing. A small number of specimens also displayed encroachment of the forewing borders into the normally yellow areas of the wings. These specific effects are presented

here under the broad categories of “Pattern Drift”, briefly described as distortions in the position and shape of the black pattern elements, and “Pattern Breakdown”, described as deteriorations and “blurring” of the pattern elements.

Pattern Drift and Pattern Breakdown can occur in the same specimen, and very often both aberrations do occur in the same specimen. However, they each occur alone in sufficient quantities of specimens to warrant treating them as two separate types of aberrations.



Fig. 3 Examples Of Pattern Breakdown Aberrations In Males And Females.

The sample specimens above are typical coldshock-induced Pattern Breakdown aberrations. The top 4 specimens (dorsal and ventral) and the first three specimens on the bottom display increasing intensities of Pattern Breakdown. Pattern Breakdown of the forewing stripes was always more severe on the dorsal surface. The second specimen on the top is the most severe stripe breakdown that was produced in PCE2. The second and third specimens on the bottom exhibit wing-wide Pattern Breakdown with displaced black scales appearing as black dusting in normally yellow areas. Such specimens have often been referred to as “melanic” aberrations over the years, although the underlying cause in our coldshocked specimens is probably different. These two specimens were produced in the PCE2 F7 generation, four generations after the coldshock procedure had been discontinued. Although no coldshock was administered after the F3 generation, these two specimens and others like them apparently acquired the ability to display this type of aberration that showed up periodically in the non-coldshocked generations. The fourth specimen on the bottom is apparently a “stow-away” yellow morph female whose ovum was inadvertently introduced on a leaf clipping. It is included here to illustrate a combination aberration that exhibits Pattern Drift in the forewing borders, Pattern Breakdown of the forewing stripes, and the semi-transparent forewing aberration caused by Insufficient Scale Quantity, all attributable to coldshock.



Fig. 4 Male Minor Abnormality Types - Stripe/Pattern Distortions

This composite photo illustrates some of the various types of stripe and pattern distortions encountered in our coldshocked male *P. glaucus* specimens. The four examples on the left show varying degrees of stripe narrowing or thickening, scalloping of the stripes due to Pattern Drift, and some “fuzziness” due to Pattern Breakdown. Additional characteristics that are noted in this classification are the extreme diminution of the outermost pattern stripes Forewing Stripe 4 and Hind Wing Stripe 2. The four photos on the right side of this plate show examples of the forewing discal stripe convergence (the left top and bottom left photos being the dorsal and ventral of the same specimen). In the most extreme cases, these converging stripes will completely encircle the yellow background scales creating a yellow Forewing Discal Cell Spot. Also of note, as can be seen in the two specimens on the right, it is possible for the forewing stripes to be extremely thickened, while the hind wing stripes remain unaffected or even diminished. In ALL cases where this abnormality occurred, the forewing stripes were always the stripes that were thickened. The hind wing stripes were NEVER the more severely thickened stripes.



Fig. 5 Male Minor Abnormality Types - Scalloped Forewing Stripes

This composite photo illustrates the progressively more severe scalloping of the forewing stripes caused by Pattern Drift. The specimen at the left is the “most normal” that this experiment produced, with its stripes being evenly black and hard-edged with no scalloping. (Note: The Forewing Stripe 2 and Forewing Stripe 4 are usually longer in most specimens.) The second specimen exhibits a minimal amount of scalloping due to Pattern Drift, and only a remnant of Forewing Stripe 4 due to Pattern Breakdown. The third specimen exhibits increased scalloping and is typical of most of our coldshocked specimens. We occasionally see a similar degree of scalloping in wild-caught specimens. The fourth specimen shows an extreme degree of Pattern Drift, with Forewing Stripe 3 merging with Forewing Stripe 4. Notice also that Forewing Stripe 1 has changed its angle, with the bottom rotating distally compared to the first three specimens.



Fig. 6 Male Minor Abnormality Types - Hind Wing Border Shading And Scalloping

This composite photo illustrates some varying degrees of hind wing shading due to “Pattern Breakdown”. The specimen on the left shows a severe scalloping of the hind wing border, which is apparently regulated or influenced by the wing veins. The three specimens to the right show progressively more shading as the yellow scales encroach what would normally be a much more solid-edged black border. Also notable is that the veins play a regulatory role here too, as can be seen in the scalloping. The border breakdown in specimen 4 is much more severe than we have ever encountered in the wild.



Fig. 7 Male Minor Abnormality Types - Black Shading Between The M_3 And CU_1 Veins

This composite photo illustrates the black shading that was occasionally observed in some specimens, both dorsally and ventrally. Here the wing veins play a regulatory role as the black shading tends to travel proximally between the veins, and in most cases, is *confined* to the space between the veins. This aberration was also seen in some female specimens.

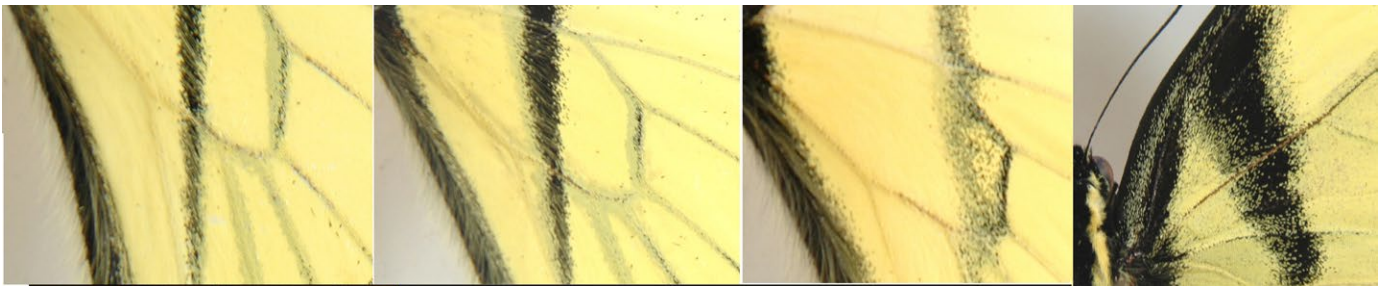


Fig. 8 Male Minor Abnormality Types - Pattern Breakdown In The Stripes

This composite photo illustrates examples of the stripes deteriorating in their intensity and boldness caused by Pattern Breakdown. The first two specimens exhibit an almost complete loss of the Hind Wing Stripe 2. The third specimen has a virtually disintegrated Hind Wing Stripe 1 that has also drifted distally by a full cell compared to the two specimens on the left. The fourth specimen shows a Pattern Breakdown in Forewing Stripe 1.



Fig. 9 Male Minor Abnormality Types - Additional Examples Of Pattern Drift And Pattern Breakdown

The two specimens at left are full wing views of some extreme male specimens produced in the PCE1 coldshock experiment. Both exhibit very similar amounts of Pattern Drift in the form of stripe scalloping. Notice that the hind wing stripes are extremely diminished, and that Hind Wing Stripe 1 has “drifted” distally. The two specimens at right both show Pattern Breakdown, not only in the degree of yellow shading caused by the deterioration of the Hind Wing Border Proximal Band, but also in the additional yellow shading in the Hind Wing Border Gap. Typical wild males vary from no blue in the Hind Wing Border Gap to possibly as many as three cells exhibiting blue. It is exceptionally rare to find a specimen with blue in more than four cells, and almost impossible to find a specimen with yellow shading instead of blue. The specimen on the right has blue or yellow shading in every cell, and is almost analogous to the pattern on the typical ventral hind wing.



Fig. 10 Extreme Pattern Drift Aberrations - One-Of-A-Kind Male Specimens

This composite shows four remarkable one-of-a-kind individuals, unlike any of the other specimens produced in either PCE1 or PCE2. Specimen 1 has an extremely narrow forewing border with slightly pointed protrusions that are aligned with the Mid-Cell Lines. It also has extreme Pattern Drift and Pattern Breakdown on both dorsal and ventral surfaces, and an almost complete absence of the normally stable hind wing stripes. Specimen 2 displays encroaching forewing borders on the ventral, prominently framing the Pistol Grip aberration. Specimen 3 displays a narrow forewing border, and is completely melanic proximal to Forewing Stripe 1. It is also missing Hind Wing Stripe 2 with Hind Wing Stripe 1 showing Pattern Breakdown. The ventral mimics the proximal melanism, and is extremely orange. Specimen 4 is the most extreme of our “melanized” males, displaying extreme melanization proximal to Forewing Stripe 1 and Hind Wing Stripe 1. The forewing apices are strongly melanized, and the hind wing borders are thickened on both surfaces.

Coldshock-Induced Aberrations in Females of *P. glaucus*

While the aberrant male specimens were quite varied in their appearance, as stated previously, they consisted almost exclusively of pattern aberrations that were created by the black pattern elements being distorted from their usual shapes and positions. By contrast, the aberrant female specimens exhibited some pattern aberrations (Fig. 2), but were overwhelmingly noteworthy for their color aberrations. These color aberrations included both displacements or distortions of the normal color patterns, and color substitutions.

The PCE1 and PCE2 experiments yielded vastly different types of color aberrations. The various female aberrations are presented here beginning with the PCE1 aberrations, next with aberrations that are common to both experiments, and finally with the PCE2 aberrations. References to the pattern map are numerous, and targeted sections of the pattern map are included for immediate reference in some of the photo plates as well. (See Pattern Maps in Supplementary Materials Section Figs. 33, 34, 35).

Aberrations in PCE1 *P. glaucus* Females

This section depicts the observed body and wing color/pattern aberrations in dark morph female *P. glaucus* in PCE1. These female aberrations consisted primarily of specimens in which the normally melanic scales were replaced by yellow scales through a process that is described in this paper as Color Reversion. Many of these yellow body and wing

aberrations have not been discussed previously. References to Groups 1-6 characteristics are depicted in Fig. 12, with an expanded representation of the groups being included in the Supplementary Materials section in Figs. 36-41.

Body Aberrations

Dark Morph Females With Yellow Abdominal Tip Ventrally

We have observed this trait many times in wild-collected dark morph females from many different populations, probably as frequently as 10% in some populations. So it is certainly a widespread, though minimally common characteristic. In our PCE1 experiments, as many as 40% of the females exhibited this yellowing of the abdominal tip. This is far in excess of what we have encountered in the wild. It is likely that this can be attributed primarily to the genetics of the original mother which exhibited this trait, but also partly to the coldshock acting to break down the melanic canalization in the F1 generation and intensifying this breakdown in the subsequent inbred generations. The coldshock-induced yellowing of the bodies seems to appear first on the abdominal tip, and encroaches forward as the overall severity of the coldshock-induced wing yellowing aberration increases (Fig. 11). **Based on our results, it is suspected that due to this tendency, wild-collected females with this trait would be more likely to produce descendents that would exhibit the same coldshock-induced yellowing effects we produced in our PCE1 experiment.**

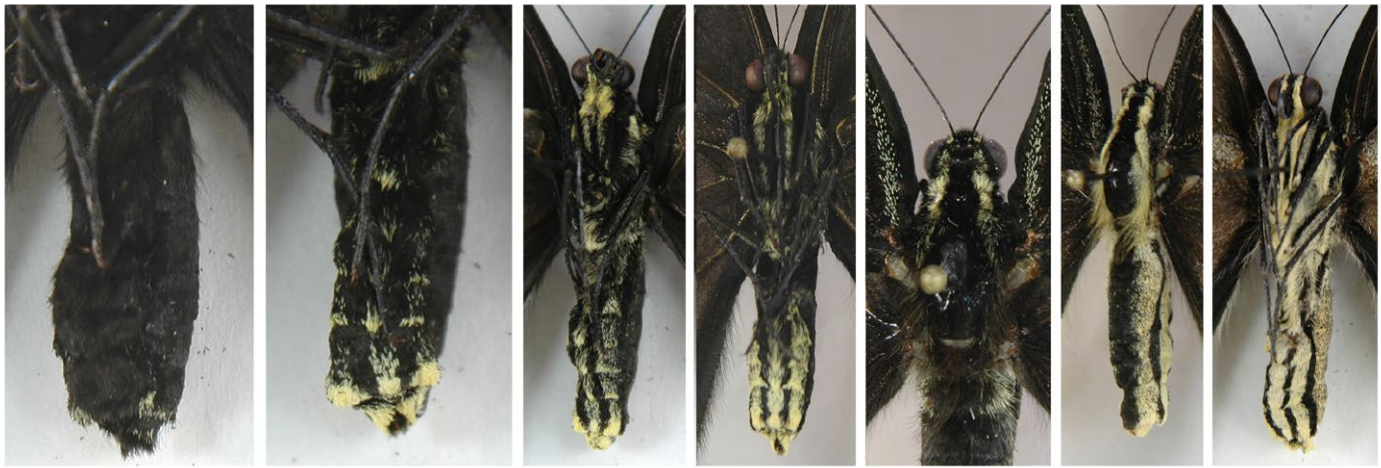


Fig. 11 Examples Of Coldshock Aberrations On The Bodies Of Dark Morph Females

This composite shows several yellowing effects that can be triggered by coldshock. From Left: Ventral view of a wild collected non-coldshocked female with virtually no yellowing at the abdominal tip; Ventral view of a typical Group 1 coldshocked female with yellowing on the abdominal tip, and very slightly on the forward segments; Ventral view of a typical Group 2 coldshocked female with significant yellowing on the entire body; Ventral view of a typical Group 3 coldshocked female showing more yellow than black on the abdomen; Dorsal view of a Group 3 coldshocked female showing yellow tufts of hair on the thorax behind the head; Dorsal view of a Group 4 coldshocked female showing the entire body now looking like a yellow morph female with all body stripes well defined and virtually no melanic dusting; Ventral view of a Group 5 coldshocked female with the entire body now being indistinguishable from that of a yellow morph female, including the legs which are now colored with yellow scales.

Dark Morph Females With Yellow Tufts Of Hair On The Dorsal Thorax

During the course of our experiments, numerous specimens eclosed with yellow tufts of hair on the thorax, like those found on yellow morph females. This characteristic started appearing in the F1 generation, and continued to appear in some specimens in all succeeding generations. As the severity of the coldshock wing yellowing increased, virtually all the specimens developed primarily yellow bodies, with the expectation that these yellow tufts would be symptomatic in accompanying the higher order of wing yellowing. It is a strikingly unexpected characteristic to see the yellow tufts on an otherwise all black body. We have never seen this trait on a wild-caught *P. glaucus* from our local population, therefore it is believed that these yellow tufts were originally triggered by the coldshock (Fig. 11).

Dark Morph Females With A General Yellowing Of The Body And Legs

It is logical to expect that the bodies of coldshocked individuals would display a proportional overall yellowing as the severity of the wing yellowing increases. Some of the bodies were quite yellow even in Group 2 specimens. As the severity reaches Group 4, the bodies are fairly indistinguishable from yellow morph bodies. By the Group 5 severity level, even the legs have become covered with yellow scales. The most interesting observation is that even when the bodies have become yellow, the adjacent proximal wing areas are often still fully melanized. The same case was true in the Ritland high temperature experiment (Ritland, David B. 1986/87) (See a brief synopsis of this paper in the Supplementary Materials section). This should be an

interesting area for future experimentation. Intuitively, it would be logical to assume that the body would be dark colored like the most adjacent wing area rather than being the opposite. Obviously, the body reverting to the ancestral yellow is caused by the same circumstances as the wings reverting to yellow, but contrary to this, the proximal wing areas are much more resistant to the yellowing. Either the body pigments are synthesized on a different timeline than the wings, or possibly the protective canalization of the proximal wing is confined to the wing and cannot protect the body from Color Reversion due to coldshock (Fig. 11).

Coldshock Induced Wing-Wide Yellowing in Dark Morph Females

In contrast to the notable but somewhat less dramatic coldshock effects described thus far, many of the PCE1 female specimens exhibited a wide variation in the background color of their wings, ranging from the normal melanic black or brown, to extremely yellowed individuals. The yellowing occurs primarily as a random distribution of individual yellow scales that have been substituted for the genetically intended melanic scales in the background areas between the stripes, thus revealing the underlying tiger stripe pattern on the dorsal surface. This dorsal yellowing is accompanied by a similar proportional yellowing on the ventral surface. On both surfaces, the yellow dusting is more pronounced distally, and is more inhibited proximally. The resulting Group 1 to Group 6 individuals (Fig. 12) are “intermediate morphs” that were caused originally by coldshock, and later by inbreeding these coldshocked intermediate morph mothers and administering additional coldshocks in each generation. The breeding choices in PCE1 were intended to intensify this yellowing, and prioritize it as the main coldshock effect being pursued. Wing-

wide yellowing occurred in every generation and intensified with each successive generation.

Starting with the individuals in the F1 generation, we originally divided these yellowed specimens into three groups based on their degree of yellowing. Three additional groups were added as the severity of the yellowing increased in the later generations, giving us a total of six groups. The parameters for each group are as follows:

- Group 1 – 5% to 20% yellow scales – Present in all generations
- Group 2 – 21% to 35% yellow scales – Present in all generations
- Group 3 – 36% to 50% yellow scales – Present in all generations
- Group 4 – 51% to 65% yellow scales – Present in F2, F3, F4, and F5 generations
- Group 5 – 66% to 80% yellow scales – Present in F2, F3, F4, and F5 generations
- Group 6 – 81% to 95% yellow scales – Present in F4 and F5 generations

The original Alto Pass mother of the F1 generation exhibited approximately 10% yellow dusting, ranking it as a minor Group 1 specimen (Fig. 1). Mothers of the F2 and F3 generations were Group 2 specimens. Mothers of the F4 and F5 generations were Group 3 specimens. Included here for convenient reference, Figure 12 shows the complete cline of

yellowed females produced in the five generations in PCE1. (Expanded examples of group characteristics can be found in the Supplementary Materials section, Figs. 36-41).

Aberrations Common To Both PCE1 and PCE2 Females

Presented in this section are two aberrations that occurred in both PCE1 and PCE2, the yellow Forewing Discal Cell Spot and the Pistol Grip aberration. Both of these aberrations are primarily attributable to Pattern Drift and Pattern Breakdown in conjunction with color substitutions.

Yellow Forewing Discal Cell Spots in Females

One of the boldest and most striking aberrations in *P. glaucus* females is the yellow spot that occurs in the center of the forewing at the discal cell edge (Fig. 13). This spot is occasionally encountered in the wild in some populations. It is exceedingly rare in our northern Illinois population, and usually when present it is faint and poorly defined. However, the yellow Forewing Discal Cell Spot is one of the first manifestations to occur caused by coldshock. Approximately 70% of our PCE1 coldshocked females exhibited this aberration, some faintly, but some with extreme boldness. The PCE2 experiment resulted in 20% to 30% of the females displaying this aberration. This yellow spot can be prominently developed even when the remaining wing is unaffected with yellow scaling from the coldshock. In general, the PCE2 specimens developed less intense yellow spots than



Fig. 12 The Complete Cline Of PCE1 *P. glaucus* Female Intermediate Color Morphs

This composite photo shows the complete series of *P. glaucus* female intermediate color morphs produced in the PCE1 series of coldshock experiments. The specimen on the far left is a typical wild collected dark morph female from Elgin, IL. The specimen on the far right is a typical wild collected yellow morph female from Elgin, IL. The six specimens in between are representatives of the intermediate color morphs that we eventually divided into Group 1 to Group 6, and constitute an uninterrupted cline of coldshock-yellowed specimens. Notice that all of the intermediate morphs exhibit the yellow Forewing Discal Cell Spot and a generally darker basal melanism. This series of specimens is a remarkable representation of what we refer to in this paper as an Aberrant Trajectory, a cline of increasing aberrant severity occurring over the course of five generations.



Fig. 13 Examples Of PCE1 Coldshock-Induced Yellow Forewing Discal Cell Spot

This composite shows a series of specimens that exhibit the yellow Forewing Discal Cell Spot. Top Row from left: A Group 1 specimen with a faint yellowing of the spot; A Group 1 specimen with a medium intensity spot; A Group 2 specimen with a very bold spot and some yellow dusting that reveals the pattern stripes. Bottom Row: A Group 3 specimen with one of the largest spots produced in the PCE1 experiment; A Group 4 specimen with a very bold spot and sufficient wing yellowing to clearly show the encircling positioning of the hard-wired stripes; The ventral view of a Group 3 specimen with a very bold spot and very converged Forewing Stripes 2 and 3.

in the PCE1 experiment.

The yellow Forewing Discal Cell Spot appears to be an obvious result of the same type of Pattern Drift that occurred in the males, specifically resulting from the broadening and migration distally of Forewing Stripe 2. Of equal interest, all five of the specimens photographed in the Ritland high temperature experiment expressed the same aberration (Ritland, David B. 1986/87). As this stripe shifts position, it closes off and encircles the area, effectively surrounding the spot with an impenetrable barrier. The yellow spot is bordered on the top and bottom by wing veins, and is bordered proximally and distally by the broadened and converged hard-wired stripes. Together these four boundaries enclose the Forewing Discal Cell Spot, in some way preventing the encircled area from being pigmented with melanin. At present, it cannot be determined why this Color Reversion from melanic to yellow occurs in this particular location. However, whatever the cause is eventually determined to be, it seems apparent that Pattern Drift is ultimately responsible for isolating this spot. Also of importance, while our PCE2 experiment demonstrated that certain aberrations delineated by Pattern Element boundaries can occur in a variety of colors, the Forewing Discal Cell Spot is yellow in ALL cases.

“Pistol Grip” Aberration at Female Forewing Apex

The dorsal forewing apex provides another opportunity for an aberration to occur, the *Pistol Grip* aberration, named for its shape resemblance to the handle of an old dueling pistol (Fig. 14). It is caused by a Pattern Breakdown in the underlying

black pattern elements. What is typically envisioned as a uniform solid black border on the forewing is actually a composite of a thin inner line that we have referred to as the Forewing Border Proximal Band, and a thick outer line that we have referred to as the Forewing Border Distal Band. These two bands are separated by an area that we call the Forewing Border Gap. Black pigmented scales normally fill the Forewing Border Gap in an uninterrupted zone, which when typically observed gives the impression of a single wide solid black border rather than two stripes with a gap in between. However, in the costalmost wing cell, the Forewing Border Proximal Band is occasionally “open” at the top, allowing a connection to be formed with the top of Forewing Stripe 4. These joined pattern elements are usually completely populated with melanic scales on the dorsal surface, but can be black with yellow centers on the ventral surface. In the extreme case where these dorsal pattern elements deteriorate, the black edges of the joined pattern elements remain intact, but allow for the change in pigment color in their center, thus creating the Pistol Grip aberration. This Pistol Grip is often present on the ventral surface of wild collected individuals, but is exceedingly rare to be even faintly visible on the dorsal surface of wild dark morph females. Numerous Pistol Grip marked females eclosed in both PCE1 and PCE2, in a variety of colors. Overall, the Pistol Grip aberration appeared as a well defined aberration on approximately 2% of the dark morph female dorsal forewings, and as a faint streak on 5% to 7%. One of the Ritland specimens also exhibits a streak in this location (Ritland, David B. 1986/87). The Pistol Grip aberration can occur alone or in conjunction with other aberrations, although it usually occurs in conjunction with

the blue trailing up the Forewing Border Gap to a greater extent than normal. In some instances the Pistol Grips are mere streaks without the “handle”, and very rarely they can be somewhat “Z” shaped like a lightning bolt. In the most boldly marked specimens, the substituted colored scales fill the area to the boundaries of the pattern elements, producing the fully formed Pistol Grip shape. Usually, the more intense the Pistol Grip, the more forewing cells exhibit the blue trailing up.

As with the Yellow Forewing Discal Cell Spot, a final explanation as to what specifically causes the color to change in the Pistol Grip is unconfirmed, but again, it is believed that the coldshock caused a Pattern Breakdown that created the opportunity for the Pistol Grip aberration to form.

Aberrations In PCE2 *P. glaucus* Females

The coldshock-induced aberrations produced in the PCE2 experiment can be divided primarily into three categories; blue-based aberrations, disproportionately lightened ventral aberrations, and insufficient scale quantity aberrations that resulted in semi-transparent wings. These three categories can be divided into subcategories that warrant separate presentation due to their repeated occurrence as individual traits throughout the experiment. It is interesting to note that the yellow Color Reversion aberrations from PCE1 did not occur to any degree in PCE2, and that the following listed PCE2 aberrations did not occur in PCE1.

Blue-Based Color Aberrations

Blue based color aberrations unique to PCE2 were produced in seven general categories:

1. Severely diminished blue on the dorsal hind wing
2. Intensified and expanded blue on the dorsal hind wing
3. “Bow Tie” Aberration
4. Blue replacing the black on ventral Hind Wing Stripe 2 at the discal cell edge
5. Blue trailing up the dorsal Forewing Border Gap
6. Intensification of the blue, distal to Hind Wing Stripe 1, referred to in this paper as the “Banded Aberration”
7. Combination aberrations including - *P. glaucus glaucus* ab. *magnificus*, **nov.**

There were also several “one-of-a-kind” specimens in which the blue was distributed differently than in these seven categories. In many individuals, several of the blue aberrations were produced in combinations, which tended to intensify all of the individual aberration types simultaneously. For clarity, the photo composites that accompany each of the described aberration types do not necessarily depict the most severe specimens that we produced. The photographed specimens were chosen because they represent as much as possible only the aberration being discussed.

Hind Wing Aberrations with Diminished Blue

There were relatively few of the diminished blue type aberrations. They are not necessarily striking in appearance by themselves, but when compared to a normal *P. glaucus* or the extreme blue aberrants, these minimal blue individuals



Fig. 14 Pistol Grip Aberration

This composite shows seven different specimens exhibiting strong Pistol Grip aberrations. The colors vary from yellowish to whitish to blue. The upper right diagram clearly shows the Pistol Grip boundaries created by Forewing Stripe 4, the Forewing Border Proximal Band, and the mid-cell line (discussed later in the Light Ventral section of this paper). A Pattern Breakdown has allowed the normally melanic scales to undergo a color substitution. Notice that the blue trails up the Forewing Border Gap in a varying number of wing cells, indicating that while potentially influential in triggering the formation of the Pistol Grip, the Pistol Grip is capable of forming independently. In addition, specimens 6, 7, and 8 also have blue in the first cell at the anal margin.

are very interesting. This aberration is occasionally seen in wild individuals, but in our local population they would be considered quite abnormal. Their common characteristics include:

1. Exceptionally dark and well saturated almost black melanic background scales which are virtually indistinguishable from the dorsal stripes
2. Virtually no dorsal blue scales proximal to the Hind Wing Border Proximal Band
3. Correspondingly dark ventral which makes the stripes difficult to see

In general, these individuals are exceptionally dark and deficient in blue on the central hind wing. Examples of the Diminished Blue aberration are shown in Fig. 15.

Hind Wing Aberrations with Extremely Intensified Blue

The first aberrant specimens that caught our attention in PCE2 were the individuals with extremely blue hind wings. We have not encountered this type of aberration previously in our local population. The blue is more vibrant than that of normal specimens in most cases. Also, there is a significantly increased density of blue scales, not only in the central hind wing, but in the Hind Wing Border Gap as well. The most pronounced of these aberrant specimens were produced in the F1 generation that was coldshocked and immediately eclosed. Their common characteristics include:

1. A high density of vibrant blue scales distributed over

most of the central Hind Wing, usually extending costally past the R_s vein

2. Most often this increase in blue scale distribution is interrupted by Hind Wing Stripe 1
3. An overall lightening of the ventral surface into the medium brown color range
4. An overall dark dorsal forewing, which in most cases can be considered normal

These individuals vary significantly in the intensity of the other wing markings. Several of these aberrant specimens also displayed a distinct lack of blue above the R_s vein, in which case the faint “sheen” which is prominent in the adjacent blue cells is also absent, revealing the normal matte-brown instead. Examples of the Extremely Intensified Blue aberration are shown in Figure 16 and Figure 17. In addition, there were a few specimens in which the blue scaling in the central hind wing was also lightly dusted onto the forewing slightly crossing the 2nd A vein.

It is most interesting to note that the stability of the pattern elements is strongly displayed in these specimens. The Hind Wing Border Proximal Band creates a strong barrier that prevents the increased blue from crossing over into the border itself. Even though the increase in blue varies considerably, there are no instances where the blue encroaches or blends into the blue patches in the Hind Wing Border Gap. Presumably, these two areas of blue in the hind wing develop contemporaneously and in close proximity, but they develop independently, strongly separated by the Hind Wing Border Proximal Band.



Fig. 15 Diminished Blue On The Dorsal Hind Wings

The sample specimens above all display the diminished blue on the dorsal central hind wing. Notice that there are virtually no blue scales proximal to the Hind Wing Border Proximal Band. Most of the specimens with this aberration display all of their other characteristics with the normal range of variation. This aberration affects only the blue in the central hind wing. The specimen on the left has extremely large Hind Wing Border Distal Band Spots, while the specimen on the right has extremely reduced spots with none of the Forewing Border Distal Band Spots on the dorsal surface. These aberrations eclosed occasionally throughout the PCE2 experiment, but were not specifically bred as part of the experiment.

Similarly, Hind Wing Stripe 1 is prominent in most of these specimens, and in some cases it functions as a barrier that impedes the formation of the blue scales proximal to it. Or stated another way, it reinforces and buffers the stability of the melanic scales proximal to it. Fig. 16 is comprised of a series of specimens from the F1 generation.

Bow Tie Aberration

Specimen 2 in Fig. 17 shows the boldest example of an aberration that we have called the *Bow Tie* aberration for obvious reasons. This aberration occurred in about 20 specimens to varying degrees, with many occurring in the F1 generation. These Bow Tie aberrations are a subset of the Intensified Blue aberration. The Bow Tie aberration has the blue concentrated in and confined to an area bordered by veins SC+R₁ and R₅, and by the Hind Wing Basal Border and Hind Wing Stripe 1. We have never seen this aberration in a wild specimen, presumably because blue is not genetically programmed to occur in this section of the wing, and also because normally the melanic buffering occurs proximal to Hind Wing Stripe 1.

Hind Wing Ventral Blue Discal Cell Stripe

The Hind Wing Ventral Blue Discal Cell Stripe is self-explanatory as far as describing what it is. We have no explanation as to why it occurs, except to say that although we have never seen it before in our local *P. glaucus*, we have seen it commonly in *P. rutulus* (Lucas, 1852) and *P. multicaudata* (W. F. Kirby, 1884). This aberration occurred in 10% to 15% of the female specimens with limited variation in intensity, and no generational statistical variation in frequency of occurrence. We did not breed specimens with this aberration; it just appeared in every generation. Specimen 3 in Fig. 17 shows a good example of this aberration on a very dark ventral surface. However, this blue stripe appeared on both dark and light ventral individuals, and on the ventrals of females where no other significant blue aberrations occurred. In no cases did the blue stripe encroach outside the normal black stripe boundaries.

Blue Trailing Up The Forewing Border Gap

Most *P. glaucus* females whether yellow morph or dark morph exhibit some blue “half-moon” shaped patches on the dorsal forewings. These occur in what we have called the Forewing Border Gap, usually occupying the first one or two cells starting at the tornus. These blue patches diminish in size and intensity as they progress up the wing, with the last adjacent wing cell in the series sometimes being occupied by only a few blue scales. Our local population rarely exhibits these blue patches in more than 2 cells, and we have never collected a specimen locally that has more than 4 cells maximum.

In some specimens produced in PCE2, the intensity of the blue diminishes in each successive patch eventually being replaced by a light creamy-yellow. The development of this

aberration requires the coldshock to break down the buffered stability of the melanic scales in this zone, thereby allowing a color substitution to occur. Notice that the breakdown is more severe at the tornus and less severe at the apex, which causes the diminishing size of these blue patches as they progress up the wing. Also, in some of our specimens the ventral forewing produced blue in the Forewing Border Gap which can be considered unusual. On the other hand, creamy-yellow Color Replacement in the ventral Forewing Border Gap is somewhat common and more visible on the ventral due to its overall lighter color. When ventral yellow Color Reversion occurs, the patches in the ventral Forewing Border Gap are normally more pronounced and often extend into every cell. Due to the essentially identical dorsal and ventral underlying black pattern elements in *P. glaucus*, it is possible that when coldshock is applied, the dorsal and ventral breakdowns in the Forewing Border Gap tend to reinforce each other. However, these breakdowns occur independently with equal frequency. (See Fig. 18)

Banded Aberration

By far the most dramatic of the blue-based aberrations we produced is the *Banded Aberration* (Fig. 19). In the simplest terms, the Banded Aberration is the result of a highly intensified color substitution that has been severely restricted between the Hind Wing Stripe 1 and the Hind Wing Border Proximal Band. Occasionally, colored scales from the Banded Aberration spill over onto the forewing. In PCE2, the primary Banded Aberration color is the normal hind wing blue. We also produced a few specimens in which the color was grayish-white instead, and we also produced a few specimens in which the color was yellow. Whichever color replaced the normally melanic scales, the pattern element boundaries remained the same. In other words, it appears that the underlying causes of the Banded Aberration are not specifically linked to the causes of either the Color Replacement or the Color Reversion pigment being synthesized.

We refer to the Banded Aberration as a dorsal manifestation, although many of the Banded aberrant specimens “mirrored” this aberration on the ventral as well, sometimes with equal intensity. These “mirrored” specimens usually exhibit only a distinct lightening of the ventral melanic background in this zone. However, some specimens exhibit a degree of color substitution as well, usually a creamy-yellow color, but in a few specimens the grayish-white color was synthesized instead. The blue pigment was not produced as a replacement color on the ventrals of these specimens.

The Banded Aberration first appeared in the F2 generation, and continued appearing sporadically in subsequent generations. In total we produced approximately 20 specimens which exhibited varying degrees of intensity of the Banded Aberration. We did not breed any of these specimens for further study.

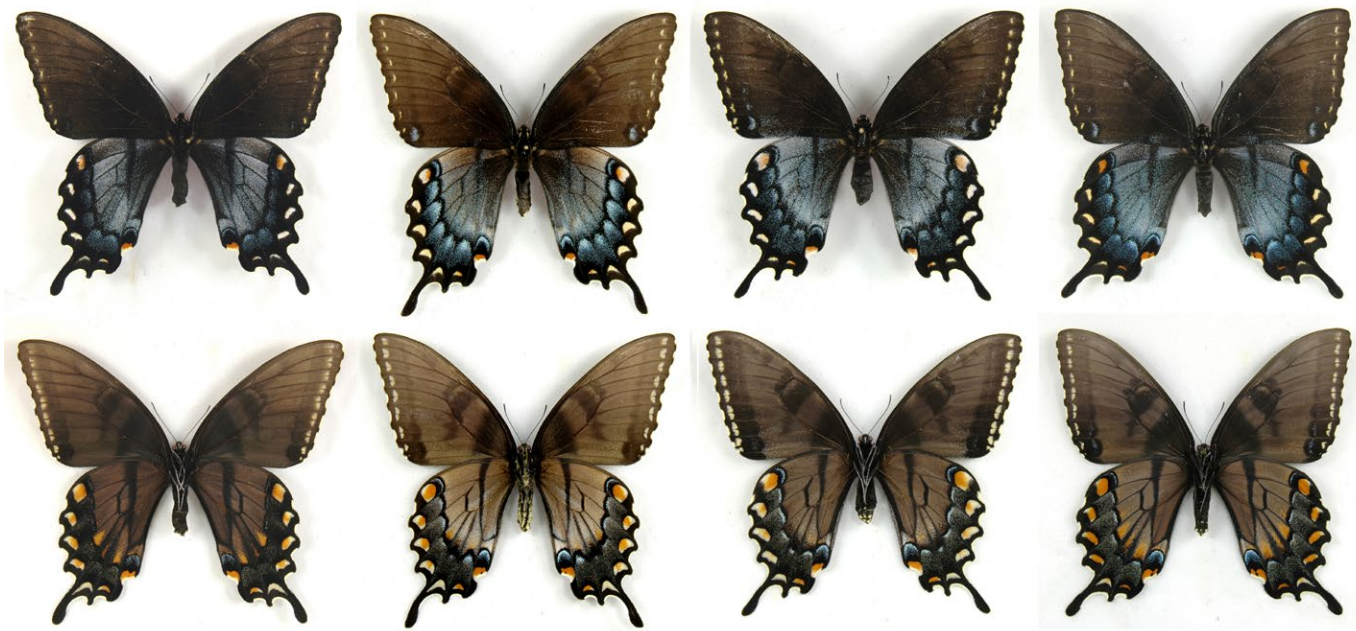


Fig. 16 Extremely Intensified Blue On The Dorsal Hind Wings

The sample specimens above show a fairly comprehensive range of variation for this “Extremely Intensified Blue” aberration. All of these specimens are from the PCE2 F1 generation chrysalides that were NOT overwintered. The first specimen in the photo displays a darkened basal zone which was similar to the yellowed specimens in PCE1. The second specimen displays a small percentage of yellow mixed in with the blue, which is unusual for this aberration type. The third and fourth specimens in this photo show progressively more blue distribution. Specimens 1 and 3 in this composite represent precursor specimens of the Aberrant Trajectory that resulted in the Banded Aberration, described later and shown in Fig. 19. They are lacking the correspondingly lightened ventral which probably intensifies and reinforces the appearance of the Banded Aberration. These specimens exhibit an extreme contrast to the diminished blue specimens depicted in Fig. 15.

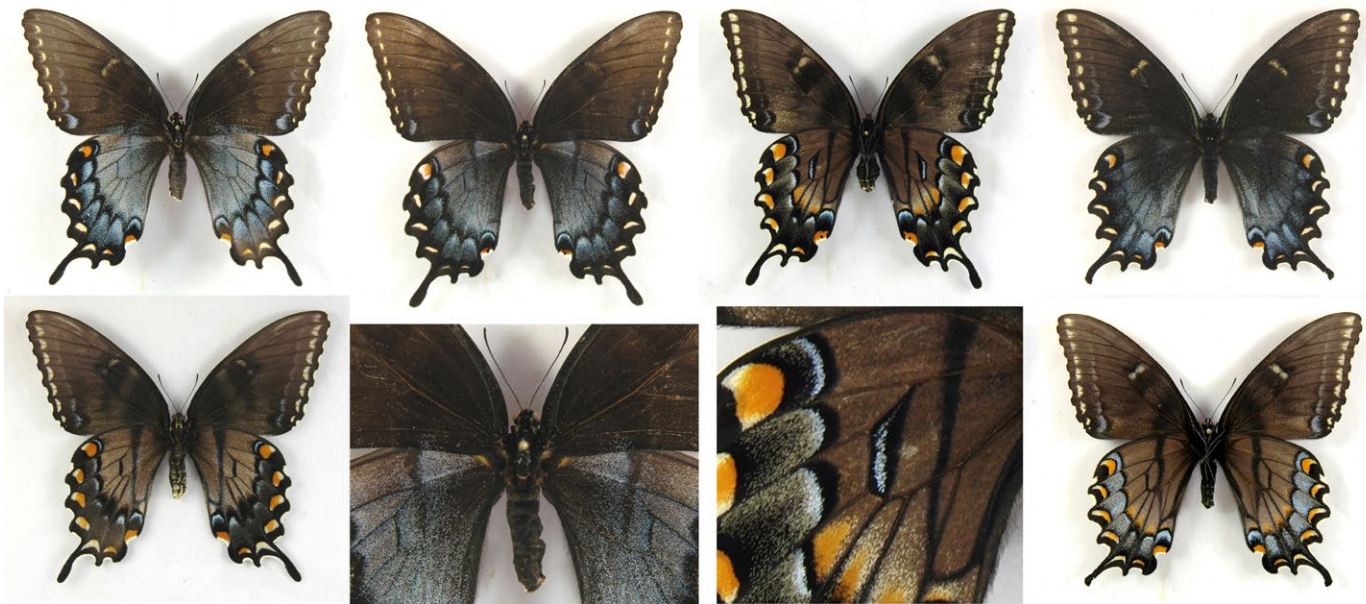


Fig. 17 Other Assorted Blue Aberrant Specimens

This composite shows four different blue aberrations. Specimen 1 is the most extensive of the Extremely Intensified Blue aberrations that occurred in PCE2. Specimen 2 shows the most intense example of the Bow Tie aberration. Specimen 3 shows the Hind Wing Ventral Blue Discal Stripe. This specimen is also interesting because the adjacent orange patches are fringed with light cream color scales. This is not normal and is somewhat of a precursor trait to what we eventually called the White Triangles aberration. Specimen 4 is a one-of-a-kind specimen with blue filling the Hind Wing Border Gap in all cells on the ventral surface. Notice that the most intense Hind Wing Border Gap cells on the dorsal are the least intense on the ventral. Specimens 3 and 4 also display the yellow Forewing Discal Cell Spot caused by Pattern Drift and Color Reversion.



Fig. 18 Blue Trailing Up The Forewing Border Gap

This composite shows four different specimens in which blue semi-circular patches trail up the forewing in the Forewing Border Gap. The first specimen is one of the relatively few that shows some of the yellowing Color Reversion characteristics that were common in PCE1. The blue semi-circles in the forewing become more creamy-yellow as they progress up the wing. The second and third specimens are fairly typical representatives of this aberration type. The fourth specimen exhibits a very strong combination of blue aberrations. It has blue trailing up the forewing in every cell, the blue Pistol Grip aberration at the forewing apex, a strongly developed hind wing blue "band", and some of this blue band encroaching onto the forewing to the 2nd A vein. Finally, notice that specimens 3 and 4 exhibit blue in the ventral Forewing Border Gap as well, while in specimen 1 the Forewing Border Gap is mostly populated by creamy-yellow scales.



Fig. 19 Banded Aberration

This composite shows four specimens that exhibit the Banded Aberration. The Banded Aberration is the result of a highly intensified Color Substitution that has been severely restricted between the Hind Wing Stripe 1 and the Hind Wing Border Proximal Band. The dark zone proximal to the Hind Wing Stripe 1 is the result of the barrier created by the strong stability of this pattern element combined with the genetic tendency of the dark morph *P. glaucus* to buffer against Color Substitution. This group of four specimens illustrates examples of the various color ranges produced in PCE2. Note that the corresponding zone on the ventral surface is lightened to severely lightened in conjunction with the dorsal aberration. We have not determined if both surfaces are linked in the formation of this aberration, but we note here that a few of our Banded specimens have more normal ventrals. Specimen 4 in Fig. 18 is a Banded specimen with a more normal ventral, and actually could be considered to be an example of aberration *magnificus* which is described in the following section. The far right specimen in this composite is an F3 female that exhibits minor mosaic patches on the left hind wing dorsal.



Fig 20. *P. glaucus glaucus* ab. *magnificus* (Perlman and Perlman 2018) nov.

This composite shows four specimens that we have assigned the aberration name *magnificus*. In total we produced eight specimens that meet our criteria. These specimens exhibit moderate to intense degrees of four aberrations simultaneously, including: Extreme Blue/Banded Blue hind wings, blue trailing up the Forewing Border Gap, Pistol Grip, and yellow Forewing Discal Cell Spot aberrations. Although the four pictured specimens vary significantly, they all display varying degrees of these four aberrant traits. Specimen 3 is the most intensely aberrant of these specimens, although two additional specimens of similar severity eclosed with severe wing emergence deformities. Of additional interest, all four of these specimens are missing the Forewing Stripe 4. Notice also that the Forewing Discal Cell Spot is yellow in contrast to the remaining three aberrations being blue. This is the result of Forewing Stripes 2 and 3 converging to enclose the spot, creating an opportunity for Color Reversion to occur, while the remaining aberrations are blue to whitish-blue resulting from Color Replacement.

***P. glaucus glaucus* ab. *magnificus* (Perlman and Perlman 2018) nov.**

In addition to the blue-based aberrations discussed thus far, we produced a series of specimens that are so incredible and distinctive that we have assigned them the aberration name *magnificus* for ease of reference. These specimens exhibit moderate to intense degrees of four aberrations simultaneously, including: Extreme Blue/Banded Blue hind wings, blue trailing up the Forewing Border Gap, Pistol Grip, and yellow Forewing Discal Cell Spot. These *magnificus* aberrations first appeared in the F5 generation - i.e. two generations after we discontinued applying coldshock to the chrysalides. So, we tentatively conclude that these specimens result from originally coldshock-induced aberrations that in conjunction with inbreeding have become heritable to some degree, and ultimately appeared spontaneously after having been recessive for a number of generations. As described above, each of the aberrant characteristics exhibited in *magnificus* can and do occur individually, or they can occur in various combinations of these aberrant characteristics. However, this is the first time we have produced specimens in which all four aberrant characteristics appeared in the same specimen. In total, we produced eight specimens in the F5 generation that meet the *magnificus* criteria, with the photographed specimens being the most striking and varied representatives.

Also at this point, it will be worthwhile to relate that the

four specimens pictured in Figure 20 are all examples of the phenomenon we describe as Aberrant Clustering, similar aberrant individuals that eclose within a narrow time window of a few hours. These specimens were refrigerated, without coldshock, on the third day after the final larval molt, and overwintered at approximately 35° to 38° F (2° to 4° C). All four plus a fifth *magnificus* specimen eclosed on the twelfth day after returning them to room temperature.

Female Aberrations - Lightening of the Ventral Surface

The previously discussed blue-based color aberrations are primarily dorsal aberrations. This section discusses the aberrant lightening that occurred on the ventral surface of many individuals. We have subdivided these ventral aberrations into two broad categories, *overall ventral lightening* and *localized ventral lightening*. The parameters of these categories are necessarily somewhat arbitrary due to the overlapping of some of the characteristics in many individuals.

Overall Ventral Lightening

It may be axiomatic that the ventral surface of *P. glaucus* is much more susceptible to coldshock lightening than the dorsal surface. No doubt, this could be anticipated partially because the ventral surface is always the lighter of the two surfaces. However, the coldshock lightening in PCE2 was often not uniform, apparently being affected both by location on the

wing surface and the stability of the pattern elements. This non-uniformity of lightening created some strikingly different aberrant individuals, as evidenced in the photographed figures that follow.

In individuals where the ventrals were minimally lightened, the hind wings usually were affected more than the forewings. In specimens where both the forewings and hind wings were lightened to similar degrees, the lightening was almost always most pronounced distally with the basal zone remaining darker proximal to Forewing Stripe 1 and Hind Wing Stripe 1 due to the same canalization we observed in PCE1. A group of specimens we classified as having overall wing-wide lightening is shown in Fig. 21.

Localized Ventral Lightening

We have identified three distinct patterns of localized ventral lightening in PCE2. To some extent, localized ventral lightening appears to be a struggle between the controlling influences of the stability of the black pattern elements, the physical barriers created by the wing veins, and the buffering tendencies exerted by the genetic stability of the dark morph evolution. A fuller discussion of these factors is beyond the scope of this paper; however, we have included the following brief commentary on wing veins as they relate to these localized ventral lightened patterns.

Influence of Venation on Some Pattern Aberrations

Wing veins influence wing patterns in numerous ways, sometimes boldly and sometimes subtly. From a developmental color and pattern standpoint, they partition the wings into individual “cells” that in some ways are autonomous. From the very narrow viewpoint of pattern analysis, wing veins can influence color and pattern distribution by preventing “spillover” of color to adjacent wing cells, or by allowing color to intensify within the boundaries of individual wing cells. Wing vein scales can even develop their own color to outline the wing cells. We have not explored how or why certain veins assert their influence, except to describe where these lightened ventral aberrations occur in relation to the veins and pattern elements. Some specific vein references are described in each aberration category, and can be understood more easily with the aid of the diagrams in Fig. 22.

Finally, we were surprised to identify *mid-cell lines* in the outer cells of the forewings. These are certainly common in some groups of Papilionids like in the *P. memnon* group (Linnaeus, 1758) and the genus *Troides* (Hübner, 1819). However, we had never seen them before in *P. glaucus* due to their faint nature. These mid-cell lines are shown in Fig. 24, and occur midway between the veins. The mid-cell lines are the only pattern elements we have observed that cross from the mid-wing into and through the border pattern



Fig. 21 Overall Ventral Lightening

This composite shows four specimens that have been lightened ventrally to a greater extent than dorsally. The two specimens at left are examples of pigmentation where the dorsal surfaces are comparable to many wild-collected specimens, while the ventrals have been moderately lightened. In addition, the ventral hind wings display an abrupt increase in lightening distal to the Hind Wing Stripe 1. The two specimens at right have been more uniformly lightened on the ventral, while at the same time showing some dorsal lightening which is distinctly yellowish as a result of Color Reversion.

elements. These were not seen in PCE1, and we have not seen them in either the yellow morph females or the males. These mid-cell lines are instrumental in causing the *sigma* aberration that is presented in Figure 24. The mid-cell lines can also cause distortions in the shapes of the various border pattern elements, such as by indenting or bisecting the Forewing Distal Band Spots. Perhaps the mid-cell lines are even necessary in the creation of the Forewing Distal Band Spots. In any case, we reference these mid-cell lines as a normally unseen additional wing pattern element, and a likely underlying influence on wing pattern development in *P. glaucus*, with possible future relevance in other species as well.

***P. glaucus glaucus* ab. *pellii* (Perlman and Perlman 2018)
nov.**

In individuals where the ventral lightening was zonal or extremely localized, three distribution patterns were the most common. The first of these localized patterns includes individuals that exhibit a lightened zone on the forewing beginning at the tornus and rather abruptly darkening at the M_3 vein, with a second lightened zone on the hind wing generally between the Hind Wing Stripe 1 and the Hind Wing Border Proximal Band. We refer to this aberration as *pellii*, named so because it reminded us of our lovable pet rabbit that was black with two white patches. Figure 23 shows a number of the more severely aberrant specimens upon which we based the *pellii*

category. It would appear that to some degree, pattern element stability also contributes to the creation of this aberrant pattern due to melanic canalization proximal to Forewing Stripe 1 and Hind Wing Stripe 1. (As a note of interest, we also produced a small number of unnamed aberrant specimens which were somewhat uniformly lightened while having more severe lightening costal to the M_3 vein. Specimen 3 in Fig. 18 is an example of this form, although we have more severe examples. The M_3 vein is apparently capable of functioning as a boundary that influences wing pigmentation.)

Aberration *sigma*

The second category of localized pattern lightening is what we refer to as aberration *sigma* for obvious reasons. The *sigma* aberration is somewhat related to the *pellii* aberration in that the forewings of both are affected in the same area. What gives the *sigma* aberration its shape is the *P. glaucus* characteristic that was not evidenced in PCE1, an underlying mid-cell line which is invisible in most specimens. Coldshock lightening has revealed that this mid-cell line is present in each of the forewing outer wing cells. In the *sigma* aberration, this mid-cell line has resisted the lightening that would normally occur from coldshock as in the *pellii* aberration, and this resistance has produced a darker wedge-shaped patch that partially bisects the pair of coldshock affected cells between the M_3 and CU_2 veins (Fig. 24).

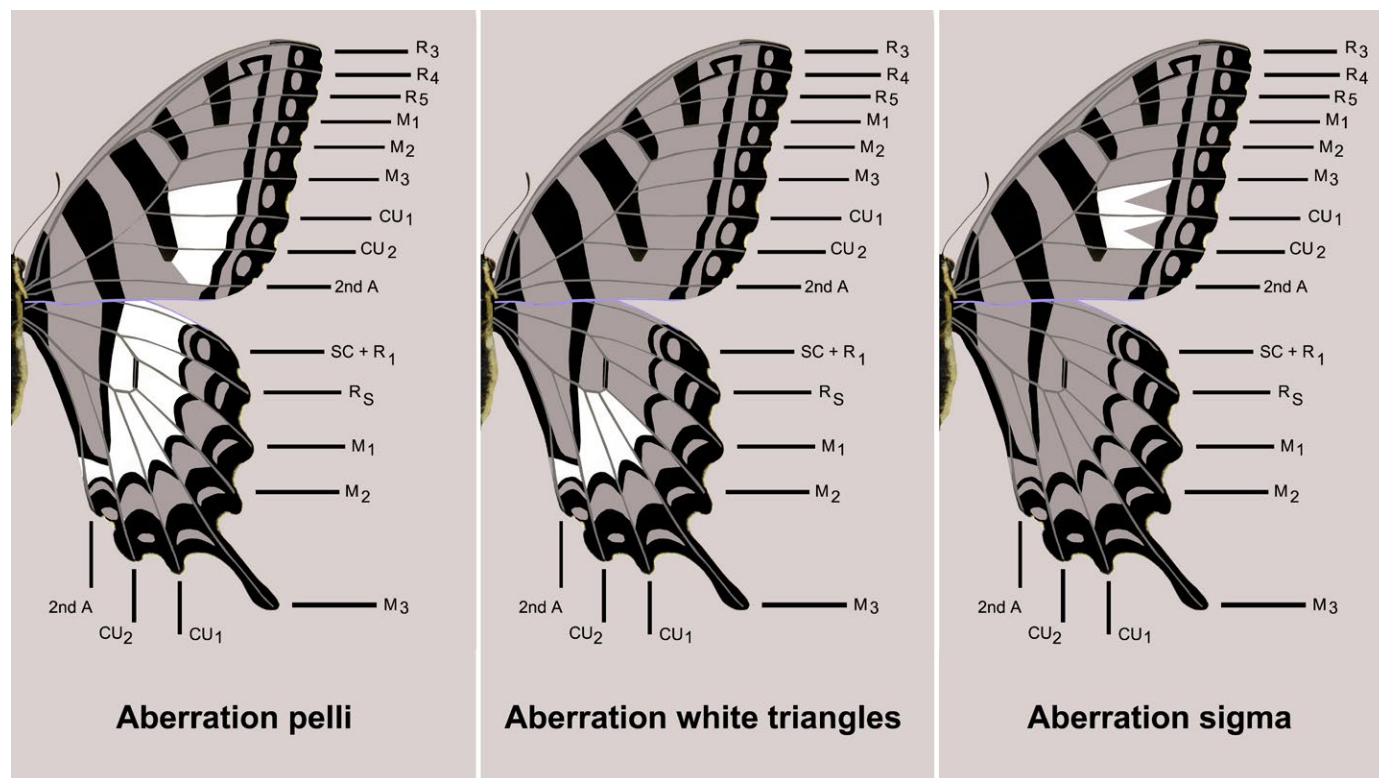


Fig. 22 Localized Ventral Lightening Pattern Maps

This composite illustrates the three types of localized ventral lightening described below. At left, the *pellii* aberration. In center, the *white triangles* aberration. At right, the *sigma* aberration. Many of the specimens exhibiting these aberrations were also combined with other coldshock aberrations, especially with the semi-transparent wing aberrations.

Aberration *white triangles*

Our third category of localized ventral pattern lightening includes individuals that exhibit what we refer to as the *white triangles* aberration. These aberrant specimens usually have more uniformly, moderately lightened ventrals of varying degrees, with one extremely lightened zone on the hind wing. The boundaries of this lightened zone are the Hind Wing Discal Cell and the Hind Wing Border Proximal Band on the

top and bottom, and beginning at vein M_2 extending to the anal margin. The *white triangles* aberration is certainly related to aberration *pellii* and the *banded* aberrations in that their locations overlap. This aberration occurred in PCE2 somewhat frequently, usually with less intensity than those specimens pictured in Fig. 25. In part, this aberration is striking because the white pigment, which we found only to be associated with blue-based aberrant specimens, is not a normal color in this location for *P. glaucus*. We also produced some specimens



Fig. 23 Ventral Aberration - *P. glaucus glaucus* ab. *pellii* (Perlman and Perlman 2018) nov.

This composite shows a range of individuals that we refer to as aberration *pellii*. Referring to Fig. 22 will make this aberration more identifiable. Notice that although these specimens are significantly lightened on the ventrals, the dorsals remain very dark. Specimens 2 and 4 also display ventral hind wing blue discal stripes. Note also that the forewing apices become distinctly darker at the M_3 vein, which appears to be the most consistent trait.



Fig. 24 Ventral Aberration *sigma*

This composite shows two prominent examples of aberration *sigma*. In addition to the *sigma* aberration on the ventral surface, these two individuals display a weaker version of this same aberration on the dorsal surface as well. Notice also that these two specimens are yellow-based (Color Reversion) specimens, with the second specimen looking like a transition form to the banded aberration. At right above is a well defined example showing the forewing mid-cell lines in all of the cells. The mid-cell line is visible crossing the Forewing Border Proximal Band, the Forewing Border Gap, and the Forewing Distal Band Spots. This is the only pattern element we have noted crossing from the mid-wing into the border. At right below is a section of the Wing Venation Map with the mid-cell lines shown in brown.



Fig. 25 Ventral Aberration white triangles

This composite shows four examples of the *white triangles* aberration. These specimens are some of the boldest and most contrasted that we produced. There were numerous specimens that were slightly less white, and several in which the orange triangular patches extended far into the white cells. Notice that the dorsals are quite dark, and the ventrals are generally minimally lightened. The white color is not a normal color found on *P. glaucus* wings, and was universally associated with specimens that tended towards blue-based rather than yellow-based aberrations.

in which the small triangular ventral orange patches are prominent within the *white triangles* wing cells.

Semi-transparent Wings Caused By Insufficient Scale Quantity

The final category of coldshock-induced aberrations presented here is the category in which wing coloration is not affected at all. Rather, the developing wings fail to produce the normal quantity of scales necessary to completely cover the wing membrane. We have called this aberration *Insufficient Scale Quantity* (ISQ). Obviously, ISQ is the result of coldshock, and we have observed it in every generation in these experiments. The exact cause of ISQ is unknown at this point. It is unpredictable in the frequency or in the severity of its occurrence. We have been unable to trace its cause to temperature, timing, lineage, humidity, or generation. ISQ individuals were produced in overwintered broods as well as in immediate-eclosion broods. Most of the individuals produced were females, but there were some males produced in the later generations as well. We have not noticed excess scales adhering to the chrysalides as if they were scuffed off during eclosion, and there are no linear rows of scales missing as if they were scuffed off from wing wear. The missing scales simply do not exist, with the presumption being that they never formed at all.

ISQ appears to be a phenomenon that is “uniformly random”. In other words, when the ISQ aberration occurs, the wings appear to be uniformly semi-transparent across the entire

wing, INCLUDING the highly stable pattern elements. However, upon close examination, the scales are distributed randomly, sometimes alternating with open spaces, and sometimes distributed in tiny clumps of adjacent scales. The scales that do form, appear to be properly formed and attached, although these scales do rub off much more easily than those of normal individuals. The ISQ aberration becomes noticeable when 10% to 15% of the scales are missing; however, we produced several dramatic specimens in which as many as 50% of the scales were absent.

Three generalizations can be made here that we believe to be consistent. First, the dorsal surface was always affected more than the ventral surface. Second, the forewings were usually more affected than the hind wings. And third, the ISQ aberration is symmetrical right to left. Occasionally, usually in the most extremely affected specimens, all four wings will be equally affected. However, we did not produce any specimens where the ventrals were more severely affected than the dorsals, or where the hind wings were more severely affected than the forewings.

While PCE1 did not produce more than a few ISQ specimens, PCE2 produced a fairly large number of ISQ specimens, possibly as many as 10% of the females. Many of the ISQ specimens eclosed with wing eclosion deformities, which may or may not be a related phenomenon. Also, there were numerous specimens in which ISQ aberrations were produced in conjunction with some of the other aberrations described in the previous sections of this paper.

Finally, two main types of ISQ aberrant specimens were produced, those individuals where only the forewings were affected (Fig. 26), and those individuals where the forewings and hind wings were all affected (Fig. 27). We have no satisfactory explanation as to why this occurred. In fact, the only tenuous explanation we have considered thus far is that

the dorsal forewings are closest to the chrysalis surface during scale formation, and perhaps being positioned outermost causes them to be more susceptible to ISQ. However, since we have not determined and isolated the environmental factors that cause ISQ, we cannot propose a hypothesis here at this time.



Fig. 26 Insufficient Scale Quantity Confined To The Forewings

This composite shows a cross-section of specimens with the aberration Insufficient Scale Quantity (ISQ), when confined to the forewings. More than twenty specimens with varying degrees of this aberration were produced. Notice that the dorsals are much more severely affected. The specimen at right is severely affected, while also being a very dark individual. Apparently ISQ effects are not linked to, or proportional to, darkness of the individual. Specimen two exhibits the most severe dorsal effects, with extremely minimal remnants of the normally stable wing pattern elements.



Fig. 27 Insufficient Scale Quantity Present On All Wings

This composite shows a group of specimens in which Insufficient Scale Quantity (ISQ) is exhibited on all four wings on both the dorsal and ventral surfaces. In contrast to the specimens pictured in Fig. 26, these individuals are much more drab in color due to ISQ affecting the hind wing color pattern. The positioning of the color pattern elements has not been altered. Notice that the normally immutable stripe and border pattern elements are severely diminished or absent. Also notice that the two specimens at right display a row of prominent darkened spots caused by the forewing Mid-Cell Lines. Finally, many of these ISQ affected specimens eclosed somewhat "crumpled". We successfully "stretched" these wings back into shape with the only indication being the "wavy" wing veins exhibited in specimen 2. Specimens 2, 3 and 4 are F4 generation specimens; they were not coldshocked. All eclosed on May 21, 2014, making them an example of Aberrant Clustering.



Fig. 28 Insufficient Scale Quantity Extreme Aberrations

The above two specimens at left are the most extreme examples of female and male ISQ aberrations that we produced in PCE2. The female at left is approximately 50% deficient in the number of scales produced, and virtually no blue scales were formed. There is a strong indication that a whitish Banded Aberration formed along with the ISQ aberration. The dark streaks on the forewings and hind wings are the result of strong stability influenced by the Mid-Cell Lines. The above male specimen is approximately 30% to 40% deficient in the quantity of scales produced. There is significant Pattern Breakdown in the stripes and border, with black dusting most noticeable on the ventral forewings. Picture 3 is an enlargement of the far right specimen in Fig. 27, showing dark patches at the Mid-Cell Lines and typical diminished scale distribution on the outer forewing. Picture 4 is an enlargement of the ventral hind wing of specimen 1 in this composite, showing typical scale distribution and also the fact that the orange scales seem to be the least affected by ISQ while blue scales are almost nonexistent.

Miscellaneous Aberrations

Wing Ecdysis Deformity Aberrations

Another interesting category of wing aberrations occurred during the course of this project. Due to the very stressful conditions caused by coldshock, we experienced a much higher than normal percentage of deformed specimens. The vast majority of these were “wing ecdysis deformities”, caused by incomplete or hampered ecdysis. In the most impacted generations, there were probably 15% to 20% deformed adults, with half of these dying as pharate individuals. Of the deformed adults that partially expanded, many of them were severely aberrant, which made their inability to expand all the more frustrating. Some of the most unique of these specimens are shown in Fig. 29.

Homeotic Aberrations - Wing Vein Anomalies

A small subset of specimens that are presented here are homeotic specimens with Wing Vein Anomalies. These include specimens with missing veins, and specimens with extra veins. Missing veins often resulted in a larger than normal wing cell accompanied by a distorted enlargement of the normal wing pattern element. Extra veins usually resulted in creating an extra wing cell where the normal wing pattern is also reproduced in the extra cell. An example of these wing vein anomalies is shown in Fig. 30. There were also numerous specimens in which partial veins developed, but because these partial veins did not completely partition the affected wing cells, no pattern aberrations resulted.

Homeotic Aberrations - Transposed Pattern Elements

PCE2 also produced two specimens in which pattern elements from the hind wing were transposed onto the forewing. We have numerous similarly affected specimens of different species in our collection, but this is the first time we have seen it in *P. glaucus*. The individual pictured in Fig. 31 is a relatively minor aberration, but we have included it because it is an anomaly produced during the course of this experiment. Transposed pattern elements would logically be caused by genetic mistakes in the developing wings. However, based on the fact that coldshock is capable of triggering a large number of different kinds of aberrations, it is entirely possible that coldshock can be a contributing factor that creates the conditions necessary for causing this aberration. Perhaps the environmental stress of coldshock reduces stability in the wing formation process enough to allow transposition mistakes like this to occur. Unfortunately, we did not have the opportunity to experiment further with this type of aberration.

ANALYSIS

When trying to analyze the causes of coldshock-induced aberrations in wing colors and patterns, we are contending with a large group of “moving targets” of an unknown number, including but not limited to: the genetic programming of the phenotype, the developmental timelines of the individuals, the availability of pigment precursor chemicals, any external influences such as temperature and humidity conditions, and a host of other unknown influences. There is no possibility of controlling all of the variables aggregately, so in many aspects



Fig. 29 Wing Eclosion Deformities With Severe Aberrations

This composite shows specimens that were severely aberrant due to coldshock effects and were also affected by Wing Eclosion Deformities. Specimen 1 displays an ISQ aberration coupled with extensive whitish-blue on the hind wings. Specimen 2 is somewhat similar, but with a more whitish-grey hind wing coloration. Notice that in specimens 1 and 2 the whitish coloration is in sharp contrast to the blue dusting in the Hind Wing Border Gap. Also, in specimen 2 the Hind Wing Distal Band Spots are abnormally white. Specimen 3 is a specimen that accidentally underwent multiple chilling and warming cycles over a several month period. It is a Banded Aberration that is almost completely blue and “satiny” in the band. Specimen 4 is an ISQ aberration that also shows an incompletely developed Banded Aberration, and has developed an extremely light ventral that is whitish rather than yellowish. PCE2 produced a large variety of these types of coldshock aberrations combined with Wing Eclosion Deformities.

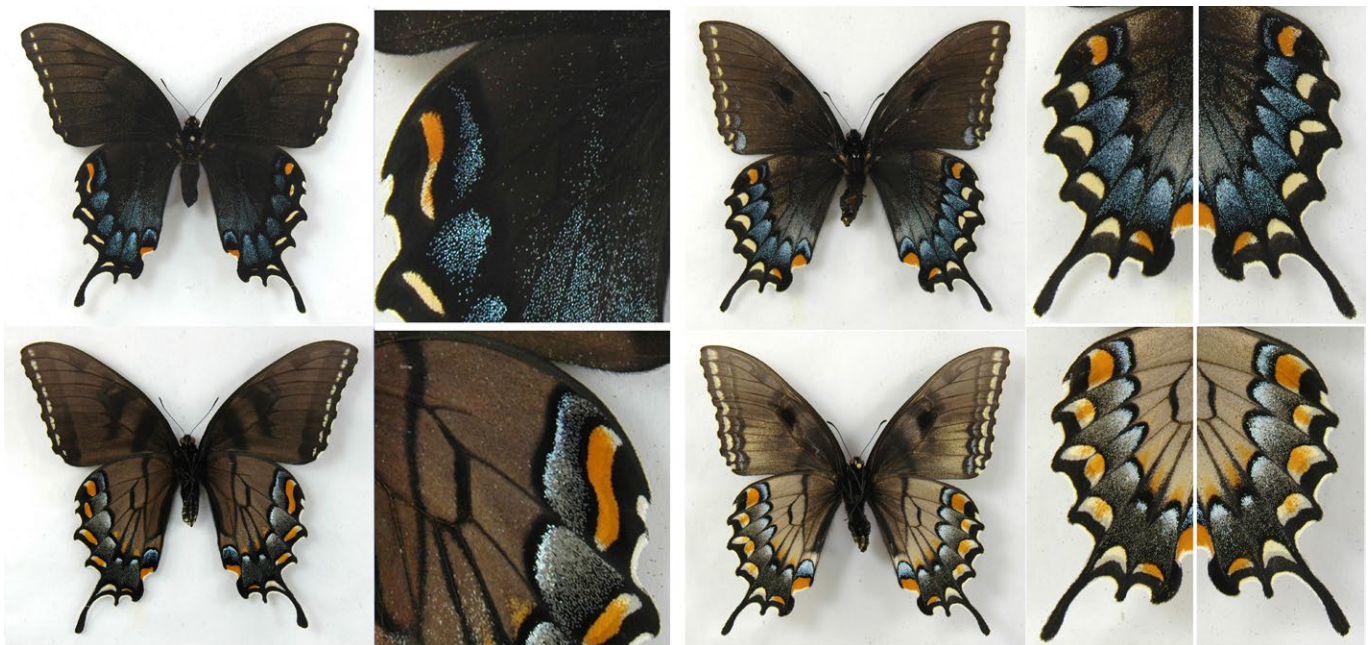


Fig. 30 Wing Vein Anomalies

In this composite, the specimen at left has a partially developed vein in the left hind wing. As a result of failing to complete its partitioning function, what would normally be two cells have been merged into one, with the crescent-shaped Hind Wing Distal Band Spots combining into one longer “S” shaped spot. The specimen at right has developed an extra vein in the right hind wing. This extra vein has caused an extra cell to form resulting in either a duplication or a splitting of the normal pattern.



Fig. 31 Transposed Pattern Elements

This composite shows a specimen in which pattern elements from the hind wing have been incorrectly produced on the forewing. This individual exhibits a relatively minor duplication compared to others we have seen, but the aberration occurs in four wing cells as indicated by the blue arrows. The upper two affected cells display what appears to be a section of the Hind Wing Border. The bottom two cells display orange from the Hind Wing Distal Band Spots that should not occur on the forewing. There are also a small number of darker melanic scales in patches that do not belong on the forewing. Otherwise this specimen appears relatively normal.

of this discussion, no definitive conclusions are possible. At this point, none of the different types of aberrations can be correlated to the specific timing of the coldshock. The differences in the severity of these aberrations could be either caused by administering the coldshock in a less than optimal window of opportunity, or by innate phenotypic capability differences between individuals.

As a consequence of producing such a vast number of different aberrations during the course of our coldshock experiments, we have devoted a lot of time and effort towards trying to “figure out” what happened that caused each of these different types of aberrations. In this section we will present speculations, hypotheses, and conclusions on various aspects of our experimental results. Much of this analysis is observationally based rather than empirically based. Some of it is based on our attempt to interpret how the various experiments of previous authors relate to our experiments (summarized in the Supplementary Materials section under “Summary of Literature Cited”). In all cases, our intention is to attempt to explain what we have observed, with the hope and expectation that future researchers will either confirm or correct our conclusions through further experimentation.

There are four main topics we examine at length in this section of the paper. The four topics are:

1. Stability of the black stripe and border pattern elements and how they are affected by coldshock
2. Color Reversion of the melanic scales to yellow

3. Four consistent “rules” governing Color Reversion to yellow
4. Color Replacement of the melanic scales by colors other than yellow

To facilitate this analysis, we have included many items in the Supplementary Materials section, including summaries of various experiments by previous authors, and additional photographs for reference. It may be helpful to refer to this section before continuing.

Stability of the Black Pattern Elements

When trying to analyze coldshock aberrations in *P. glaucus*, one of the most fundamental concepts we recognized is the extreme stability of the black stripe and border pattern elements. These pattern elements are normally extremely resistant to disruption and they demonstrate exceptional stability in position, saturation of pigmentation, and especially in the color being *always* black. Not only are they highly resistant to alteration by coldshock, but they also exhibit a high degree of influence over the formation and visual appearance of various coldshock effects across the entire wing surface.

Visual Effects of Pattern Drift and Pattern Breakdown

Despite the innate stability of the black pattern elements, there were some individuals that did display coldshock-induced alterations to the stripes and borders. The most visible distortions in the stripe and border pattern include stripe dislocation, stripe extension or shortening, stripe scalloping, stripe diffusion where the boundaries are softer or more diffuse, stripe widening or narrowing, border encroachment, and border deteriorations in various locations. These specific effects are discussed in this paper under the broad categories of Pattern Drift and Pattern Breakdown.

We have used the term *Pattern Drift* in this paper to describe minor intact dislocations and migrations of the various black pattern elements to slightly displaced positions on the wing surface. Pattern Drift is most frequently seen in the forewing stripes in the form of “stripe scalloping”. This stripe scalloping is very common and even typical in wild-collected individuals. However, many of our coldshocked specimens seem to exhibit this effect more severely. Also included in the category of Pattern Drift are severe thickening or narrowing of the pattern stripes, and occasional encroachment of the Border Proximal Bands proximally (Figs. 2,4,5,7,8,9,10).

We have used the term *Pattern Breakdown* in this paper to describe a generalized or localized deterioration of the pattern borders and stripes, in most cases creating a fuzziness, a faded appearance, or a blurring effect in the normally hard-edged pattern elements. In other instances, especially in the dorsal borders, there sometimes appears a dusting of yellow, blue, or cream colored scales in the center of the normally very solid black areas (Figs. 3,4,6,8,9).

Pattern Drift And Pattern Breakdown Aberrations

Over the course of our experiments, five Pattern Drift and two Pattern Breakdown abnormality types were identified. The most common Pattern Drift aberration to occur was “scalloping” of the forewing stripes, most easily seen in males, caused by what would appear to be an “outward pressure” which is more intense at the wing veins. This gives the stripes a distinct “windblown” appearance (Fig.5). Second, a few of the male specimens varied from our typical local wild-caught specimens to a greater extent in the thickness of the black stripes, with stripes in some specimens being noticeably wider and in some specimens being noticeably narrower than we usually encounter (Figs. 2,4,). The third common Pattern Drift aberration was the convergence of Forewing Stripes 2 and 3, caused by Forewing Stripe 2 migrating distally, and often with both stripes being wider than normal. This stripe convergence often encloses the yellow background scales, most easily seen in the dark morph females, creating a yellow Discal Cell Spot instead of allowing the normal contiguous connection to the remainder of the wing surface (Figs. 2,4,13). The fourth Pattern Drift aberration we identified was that some specimens had an intrusion of black scales between the caudal veins CU_1 and M_3 on the hind wing (Fig. 7). The fifth and most infrequent Pattern Drift aberration was the encroachment of the Forewing Border Proximal Band proximally into the background colored scales, sometimes connecting with Forewing stripes 3 or 4. There were also a few specimens in which disconnected black spots occurred in the forewings (Figs. 2,10).

Pattern Breakdown occurred fairly frequently in the male specimens. Many individuals displayed the most severe Pattern Breakdown in Forewing Stripe 1, with some specimens showing more than 50% diffusion of the stripe. In other individuals, the stripe deterioration occurred to some degree in all of the stripes. In some individuals, the outermost stripes were missing or extremely diminished. In addition, a few specimens displayed Pattern Breakdown of the black forewing and hind wing borders (Figs. 3,4,6,8,9,10).

All of these Pattern Drift and Pattern Breakdown aberrations can be attributed to the coldshock, but it must be noted that we occasionally see similar effects in wild caught specimens, albeit to a lesser degree and with less frequency.

Pattern stability also normally exists in the dark morph females, even when the black stripe and border pattern is not visible due to the darkness of the background melanic color. In the darkest females, there is no indication of the underlying pattern on the dorsal surface, and only very minimal visibility of the pattern on the ventral surface (Figs. 1,12). In the lighter of the normal dark morph females, the underlying pattern will be minimally visible on the dorsal surface, and very evident on the ventral surface. When present, this visibility of the pattern elements in *normal* dark morph females is the result of the melanic background wing scales being colored chocolate-brown instead of black, and is not due to pigment color

substitutions in the background scales.

So the two questions pertaining to the black pattern elements that arise are:

1. *Why does coldshock not materially affect these black pattern elements either in color or intensity?*
2. In the Koch experiments (Koch, P. B., et. al., 2000a), (see Supplementary Materials section, Background - Research By Previous Authors), both the black pattern elements and the melanic background scales of the females matured and were pigmented contemporaneously, suggesting that both the black and brown melanic pigments share the same origin. *If pigmented in the same melanic timeline window, why are the pattern elements always black while the background melanic scales can be various shades of brown?*

Pattern stability has been previously described in other butterfly species, and there are two series of experiments that seem particularly relevant to our experiments. The first was a series of coldshock experiments performed by Nijhout on *Vanessa* and *Precis* Nymphalids. He noted higher stability in the proximal half of the *Vanessa* wings (Nijhout H. F. 1984), and higher stability of the forewing discal red bars in *Precis* specimens (Nijhout, H. F. 1980a). Pertaining to the red bars, he concluded that their stability is probably due to the fact that these pattern elements have already been determined by the time the temperature shock is applied.

The second experiment of relevance, performed by Koch, et. al., pertains to color stability in the black vs. brown melanic pigments in the Satyrid *Bicyclus anynana* (Butler, 1879). They stated that, “The black scales of the eyespot initiate visible melanin synthesis earlier than the dark-brown scales in the background, leading to a darker (black) final color.” (Koch, P. B., et. al., 2000b). A similar scenario probably exists in *P. glaucus*.

So, based on these precedents, it is logical to assume that the innate stability of the black pattern elements is the result of an earlier fate determination that precedes the coldshock, accompanied by an earlier initiation and extended window of melanin synthesis in these scales. For the purposes of discussion here, we have used a “hard-wired” vs. “programmable” comparative model. In this model, even though the pattern stripes and the melanic background scales of the dark morph females are melanized in the same timeline window, the black borders and tiger stripes are “hard-wired” components of the overall wing color/pattern template, based on the assumption that their fate determination occurs much earlier in time than that of the melanic brown background scales. As a result, the black pattern elements are not easily altered by coldshock, because their fate determination is already established *prior to the initiation of the coldshock procedure*. At the same time, the background melanic scales act more like “programmable” entities, with the assumption being that their fate determination occurs later in the developmental timeline, probably near the time of coldshock

initiation. Due to their lack of prior fate determination, the background scales have the capability of changing their color or saturation levels to conform to the biological or environmental circumstances to which they are subjected. This “hard-wired” vs. “programmable” model is very advantageous when trying to analyze coldshock effects in *P. glaucus*. It is hoped that further experimentation will test the efficacy of this model.

Color Reversion Of Melanic Pigmented Scales To Yellow Pigmented Scales

This section discusses the most dramatic manifestation of our PCE1 coldshock experiments, the substitution of papiliochrome yellow pigmented scales for the genetically programmed melanic background scales – i.e. transforming a dark morph *P. glaucus* into a yellow morph *P. glaucus*. This analysis is presented in two generalized aspects: first, the overall yellowing that occurs dorsally and ventrally, and second, some observed peculiarities pertaining to the inconsistent manner in which the yellow is distributed across the wing surface. (For readers less familiar with the species *P. glaucus*, previous authors’ wing pigmentation experimentation, and previous authors’ coldshock experimentation, it would be highly beneficial to first read the “Summary of Literature Cited” in the Supplementary Materials section for some requisite background information and quick reference to the authors referenced in this section.)

Wing-wide Color Reversion

As stated at the beginning of this paper, *P. glaucus* is very unique among the Swallowtail butterflies. First, its two female morphs are virtually identical in their hard-wired stripe and border pattern with the only difference being either the melanic brown or the papiliochrome yellow color of their surrounding background scales. Second, the basic black pattern elements of *P. glaucus* are essentially identical on the dorsal and ventral wing surfaces. And third, its two morphs exist sympatrically in relatively similar ratios throughout the entire distribution range, in contrast to many dimorphic butterflies that exist as nonintersecting regional forms.

Given that the P. glaucus females are genetically programmed so that yellow morph mothers produce yellow morph daughters and dark morph mothers produce dark morph daughters, how is it possible for the melanic background scales to become yellow without a genetic mutation being responsible? The short answer may be that there does not need to be a genetic mutation because the ability to change from brown to yellow already exists due to the shared ancestry of the two morphs. The Koch experiments demonstrated that chemically, the melanic pigments and the yellow pigments are both derivatives of similar initial chemical components, and that via the presence or absence of the proper enzyme catalyst, these initial chemical components can become either color (Koch, P. et al. 1998). This shared chemical origin probably explains how the dark morph could have originally evolved

from the yellow morph, and why the melanic scales of the dark morph are prone to default to their ancestral yellow color. Hence, coldshock somehow circumvents the individual scale’s genetically programmed brown pigment selection by forcing it to choose the ancestral yellow as the “default” pigment selection.

We would characterize this color substitution as “Color Reversion” rather than “Color Replacement”. Color Reversion acknowledges the ancestral origin of the yellow pigment and its chemical relationship to the melanic brown pigment. We use the term Color Replacement extensively in analyzing our PCE2 specimens, where we explain that the various color substitutions in PCE2 are chemically unrelated to the melanic pigment they are replacing.

Is it possible to theorize an explanation as to how this Color Reversion occurs? Starting from the assumption that a mature *P. glaucus* wing scale MUST be pigmented, and that due to the ancestral chemical relationship, the two primary options for the background scales are either yellow or brown, the following parameters apply: Under *normal* circumstances, Otaki’s 4-stage model provides us with the sequential stages of how the wing scales choose which pigment to synthesize (Otaki, J. M. 2008). Koch tells us that when a wing scale’s color has been determined via the Otaki 4-stage process, it must mature to the proper extent to enable it to synthesize its pigment (Koch, P. B., et al 2000b). Koch continues that the scale maturation rate is genetically synchronized with the window of availability of the correct precursor chemicals that enable the synthesis of the proper color. This enables the scale to successfully carry out its genetically predetermined instructions. And finally, Koch tells us that yellow always precedes brown in the pigmentation sequence (Koch, P. B., et. al 2000a & 2000b).

Next, Koch has determined that the same precursor chemicals can either be used to synthesize yellow or brown, and the Koch experiments have provided us with the step by step process that makes this selection possible (Koch, P. B., et. al., 2000a). The first important *intermediate* step in the pigment synthesis process is that the enzyme dopa-decarboxylase (DDC) facilitates the production of dopamine. Dopamine is a critical substance used in the production of both the yellow and brown pigments. The next important step is the production and activity level of the enzyme N-β-alanyldopamine synthase (BAS). The dopamine is acted upon by BAS to produce papiliochrome yellow or *not* acted upon by BAS to produce melanic brown. Koch demonstrated that high concentrations of BAS cause the dopamine to be used in the production of yellow, and low concentrations of BAS cause the dopamine to be used in the production of brown.

So, now that we know *how* the Color Reversion is made possible chemically, the next question is: *Why did our coldshock cause this Color Reversion to occur?* The answer is probably that the initiation of the coldshock procedure within the first three to five hours after pupation causes a disruption

in the Otaki 4-stage process (Otaki, J. M. 2008), thereby overriding the dark morph genetic programming, and forcing a change in the fate determination to yellow. The remarkable thing is that this apparently occurs on a scale by scale basis rather than on a wing-wide basis. This may be observed in some specimens as a “graininess” in the scale distribution, and by the generally “salt and pepper” overall intermingling of the yellow and melanic scales. The continued yellowing in subsequent generations was probably caused by a combination of coldshock, inbreeding, and selective breeding to favor this yellowing.

Assuming that a disruption in the Otaki 4-stage process is the cause of the Color Reversion to yellow, there is one additional aspect to this theory that must be considered. The Ritland experiment demonstrated that overheating during the larval and pupal stages also yields similar looking Color Reversion adults (Ritland, David B. 1986/87). Because both overheating and coldshock cause *environmental* Color Reversions rather than *genetically mutated* color changes, the ultimate underlying cause must be susceptible to both temperature extremes.

Non-Uniformity Of The Color Reversion

The second aspect of the Color Reversion phenomenon that is especially interesting is the fact that the yellowing is not uniform across the entire wing surface. While there is a proportional degree of yellowing on the dorsal and ventral wing surfaces, the proximal and distal areas of the wing surfaces are not equally affected. The proximal areas are much more resistant to the Color Reversion, and also in

some specimens the yellow scale distribution has a distinctly “granular” appearance. We offer for comparison the photos of two hybrid female specimens that display a far more uniform color distribution (Fig. 32). The hybrids in the photo are crosses that we made between a *P. multicaudata* male and a dark morph *P. glaucus* female. Both specimens eclosed naturally without chemical injections of any kind. These mixed-pigmented hybrid specimens are totally *genetic* in origin, and provide a remarkable contrast to our coldshocked *P. glaucus* specimens which are at least partially *environmental* in origin. The hybrids apparently do not have the proximal buffering ability, presumably because the *P. multicaudata* component of the hybridized genome never needed to develop dark morph canalization due to its females being monomorphic yellow.

Four Consistent Tendencies Of Coldshock-Induced Color Reversion In *P. glaucus*

After exhaustive examination of our PCE1 female yellowed specimens, we have concluded that there are at least four consistent tendencies governing coldshock-induced Color Reversion in *P. glaucus*.

Rule 1: Color Reversion yellowing of the wings is more pronounced distally (Fig. 12, Supplementary Materials Figs. 36 - 41). In our experiments, this can be considered axiomatic, because in over 1,000 specimens being bred during PCE1 and subsequent experiments, all of the females exhibited more pronounced distal yellowing and none of the specimens deviated from this rule. The same distal yellowing was also shown in the Ritland photographs (Ritland, David



Fig. 32 Comparison Of Melanized Scale Distribution In Coldshocked vs. Hybrid Individuals

This composite photo illustrates the differences in the melanization pattern exhibited in coldshocked individuals and hybrid individuals. From left: a PCE1 Group 4 individual dorsal and ventral; a PCE1 Group 5 individual dorsal and ventral; a female hybrid of a dark morph *P. glaucus* female and a *P. multicaudata* male eclosed in 14 days; a female hybrid from same brood after overwintering in diapause. Aside from the hybrids being generally more orange, notice that there is very little variation in the overall distribution of the melanic scales. Presumably this is because they are genetically darkened from the *P. glaucus* genes and randomly produced across the entirety of the wing surface. By contrast, the coldshocked *P. glaucus* individuals exhibit the typical “gradient” type of mid-wing and distal yellowing, and basal melanism, presumably caused by developmental timing, melanic canalization, and/or the “diffusion barrier” created by the stripe pattern.

B. 1986/87). In most specimens there is a slight gradient in the Color Reversion from the mid-wing outward. There may be a number of explanations for this phenomenon. Based on Nijhout's cautery response experiments, perhaps the options he presented can be extended and applied here (Nijhout, H. F. 1985a).

1. *Wing surface development is asynchronous.* This option can be extrapolated to propose that either the wing scales commence their *color determination* distally and proceed proximally, or the wings initiate *pigment synthesis* distally and proceed proximally. Both of these circumstances require the presence of ample amounts of BAS.

2. *Wing surfaces differ in their susceptibility threshold* positionally. It is possible that concentrations of BAS vary from location to location on the wing membrane. This option requires either initially higher concentrations of BAS distally, or possibly a slower dissipation rate distally. Either of these scenarios would result in a more lengthy yellow "pigmentation window" distally.

There are probably several additional possibilities that are not presented here.

Rule 2: The second consistent tendency is a corollary to Rule 1 and is perhaps the most impactful. **The basal zone is significantly darker than the mid-wing and distal zones on Color Reversion type coldshocked females.** This effect is apparently caused by the relatively immutable Forewing Stripe 1 and Hind Wing Stripe 1. In many specimens this basal dark zone is abruptly terminated at these stripes (Fig. 12, Supplementary Materials Figs. 36 - 41). Based on the premise proposed earlier in this paper regarding pattern stability, these hard-wired pattern stripes create a "diffusion barrier" on the wing surface. These are not structural barriers like the wing veins. These particular stripes traverse several wing cells at perpendicular angles and would not pose an obstacle to chemical flow if the veins were the conduit. As previously stated, these hard-wired stripes presumably receive their programming and establish their stability at an earlier point in time than the remaining wing scales. Because these stripes extend vertically across the entire forewing and hind wing, they are capable of inhibiting the chemical flow across the wing surface. This basal melanized zone only showed the first indications of Color Reversion in the PCE1 F2 generation and later. Apparently the melanic canalization capacity in the basal zone is diminished by inbreeding coldshocked parents that subsequently transmit a decreased canalization capacity to their offspring. This diminished canalization ability was then accentuated by coldshocking the offspring in subsequent generations.

There are several factors that could, as a consequence of the proximal stripe diffusion barrier, contribute to the formation of the darker basal zone. The most logical starting point is that in this zone there is a relative deficiency in the levels of BAS, at

least at the time of pigment synthesis. Three possible causes may be at play here:

1. Coldshock is *unable* to substantially increase BAS levels in this zone, thereby facilitating the scales' existing ability to synthesize the proper genetically programmed melanic pigment.
2. Coldshock is *unable* to accelerate scale maturation rates in this zone, thereby allowing the scales to mature in the "melanin synthesis window".
3. Coldshock is *unable* to disrupt the Otaki 4-stages in this zone, thereby allowing these scales to proceed along their genetically predetermined developmental path.

So the question becomes, *What capabilities and functions do the pattern stripes possess that allow them to keep the basal zone melanized?* First, Forewing Stripe 1 and Hind Wing Stripe 1 are the only stripes that span the entire forewing and hind wing, effectively preventing chemical diffusion (or possibly fate determination) in either direction across the barrier. Second, the fate determination of the pigmentation for these stripes preceded that of the background scales and also evidently preceded the application of the coldshock. Third, perhaps the stripes themselves act as foci for chemical inhibitors that keep the basal zone canalized against Color Reversion. More experimentation is necessary before a definitive answer to this question can be offered.

Rule 3: **The dorsal and ventral wing surfaces undergo a relatively proportional yellowing with the ventral being lighter.** There were no specimens produced in which the dorsal surface was lightened more than the ventral surface. This was to be expected because the ventral is always the lighter surface whether dark or light morph, or whether wild-caught or coldshocked. However, we note that the dorsal surfaces of coldshocked females have a "salt and pepper" type of scale distribution character, with adjacent scales being either fully saturated brown or yellow. In contrast, the ventral surfaces are often more uniform in pigmentation, looking more like the overall lightening is a blend of yellow scales mixed with poorly saturated melanic scales. There is no hypothesis given here at this time to account for this difference in appearance, other than to state that the ventral surface is always inherently the less vivid of the two surfaces. Perhaps the coldshock has the ability to affect the pigment saturation level attainable by the individual scales.

Rule 4: **The bodies are pigmented independently from the wings,** at least temporally. There is no indication that the bodies are in any way affected by the reversion resistance of the adjacent dark basal wing zone. The bodies can be totally yellowed like a light morph *P. glaucus* with the immediately adjacent darkened basal zone being fully melanized. Apparently, what happens on the wings stays on the wings.

Color Substitution Aberrations In Dark Morph Females

Color Reversion vs. Color Replacement

Our coldshock experiments have demonstrated that the predetermined wing color templates that are genetically established in the developing embryo, can be disrupted and reprogrammed during pupation to result in radically different color substitutions that are contrary to the original genetic programming. This became increasingly obvious in our PCE2 experiment, because the color aberrations in PCE2 were drastically different from the wing-wide yellowing in PCE1. In an attempt to explain how these radically different types of color substitutions can occur, we have divided these color substitutions in *P. glaucus* into two categories, *Color Reversion* and *Color Replacement*.

In PCE1, the primary type of coldshock aberration to occur in the dark morph females was a *Color Reversion* of the intended melanic brown background scales to papiliochrome yellow. The causes of this Color Reversion were discussed above and were postulated to be a combination of: coldshock disruption of the 4-stage scale color determination process (Otaki J.M. 2008), the unique chemical mechanism that allows melanin production to be diverted to papiliochrome yellow production (Koch et al 2000a), and the premise that in *P. glaucus* the underlying ability to change to yellow is innate and is the result of the yellow morph being the genetically ancestral default color. Our definition of Color Reversion requires that the two pigments involved (yellow and brown) must be chemically related as “either/or” derivatives of the same precursor chemicals. The black pattern elements remained relatively stable and were not affected by Color Reversion.

In the PCE2 experiments, the primary color changes that occurred were either blue-based or white-based aberrations. So, instead of Color Reversion being the main coldshock manifestation, the color substitutions in PCE2 were caused by *Color Replacement*, characterized as such because the blue and white pigments are *not* chemically related to either the yellow or melanic pigments. A wide assortment of aberrant color patterns were produced in PCE2, primarily as the result of incorrect colors being synthesized and filling in “gaps” between the black pattern elements in the locations where either the yellow or melanic pigments would normally have been produced. Once again, the black pattern elements remained relatively stable and were not affected by Color Replacement.

All of these various types of color aberrations indicate that there is much more flexibility in pigmentation and pattern creation than previously imagined or demonstrated. *Some* of these aberrations are made possible by biochemical inter-relationships between pigment colors, but *all* of them are caused by reprogramming the developing wing scales to synthesize the wrong colors, facilitated by the application of coldshock. In the broadest sense, in addition to genetic programming, the synthesis of wing color pigments is

dependent on three factors: the availability of various pigment precursor chemicals, the maturation status of the individual scale, and which color pigmentation window is active at the time of scale maturation. In normal development, these factors are genetically synchronized, so that the correct wing pattern will be properly implemented. In our aberrant specimens, this original genetic programming is overridden and nullified by some combination of disruption by the stressful coldshock circumstances that cause breakdowns in the original programming, and subsequent selective breeding that enhances suppressed phenotypic proclivities.

COMMENTARY

As evidenced by the specimens photographed in this paper, the breadth and scope of the types of aberrations occurring in *P. glaucus* made possible through the use of coldshock are truly enormous. Most of the aberrations produced during the course of our experiments are not seen in the wild. Some of them have never been seen prior to our experiments. However, we have demonstrated that once these aberrant tendencies are produced and identified, through the use of selective inbreeding and additional coldshock, they can become heritable to some degree, and are even capable of being intensified in subsequent generations. Also, the two experiments we conducted, PCE1 and PCE2, have suggested the possibility that the genetic peculiarities native to different populations of *P. glaucus* can result in dramatically differing reactions to identical coldshock techniques. Despite this vast array of possible coldshock-induced aberrations, some commonalities exist. Most notably, the innate stability of the black pattern elements evidenced over the course of both experiments. While the relatively few exceptions caused by Pattern Drift, Pattern Breakdown, and ISQ disrupted the overall appearance of the pattern, the black pattern elements never exhibited a change in color despite major color changes in adjacent areas of the wing.

The explanation as to why the black pattern elements cannot be induced to change color while the adjacent background melanic brown scales can be affected by either Color Reversion or Color Replacement, was addressed above. The conclusion forwarded was that the black pattern elements receive their fate determination prior to the coldshock being administered, and therefore they are precluded from subsequent alterations to their programming. A similar stability observation can be made about the orange pattern markings. Having reared over 2,000 individuals over the course of PCE1 and PCE2, there were no widespread instances where the orange markings noticeably exceeded those of wild-collected individuals. The obvious, though tentative conclusion, is that our coldshock technique is incorrect in either temperature or in timing to cause a disruption in the orange pigmentation process or its underlying fate determination.

Single Scale Identity

Over the course of our experimentation, we have considered the possibility that the majority of individual *P. glaucus* background melanic colored wing scales can function as a blank slate, by virtue of their lack of previously established fate determination. Once freed by coldshock from their genetically intended melanic programming, they are capable of being reprogrammed and thereby pigmented virtually any color, in complete contradiction to their original genetic programming. As a result, these scales exercise their own individual “single scale identity”. In other words, once the original genetic color template has been disrupted by coldshock, it appears that the pigment selected by each individual scale is dictated by its incorrectly implemented fate determination. This causes the scale to mature in the different color window that is appropriate to its new programming and synthesize the incorrect substituted color (Koch, P. B., et. al. 2000a & 2000b) (Nijhout, H. F. 1980a). (This may also represent a possible explanation as to why “out of place” alternatively colored scales are formed in otherwise solid color sections on many Lepidopteran wings). If, in *P. glaucus*, the different colored wing scales are identical in their formation, differing only in their fate determination and their resulting pigment color synthesis, it may not be difficult for them to function as if they were a blank slate.

On the surface, this seems to defy the logic that a butterfly must maintain its pattern identity in order to retain its species identity. However, in our series of experiments, the genetically brown dark morph *P. glaucus* has demonstrated that its brown hereditary programming can be overruled to instead synthesize yellow, blue, orange and white. As far as we have observed, the only limit on the selection of which substituted color will be synthesized is that the color must exist within the normal color palette of the dark morph *P. glaucus*. Further, these substituted colors, at least in the F1 generation, are *not* incorrectly genetically programmed in the embryo as mutations, but are the result of *reprogramming* in the chrysalis on a scale by scale basis.

Aberrations In Multiple Colors

The female color aberrations produced in PCE1 and PCE2 encompass a wide variation in color distribution and intensity. In most cases, an aberration's outlines are determined by the stability of the pattern elements, and only in rare instances did a failure of pattern element stability create an opportunity for the color to “spill over” outside the boundaries of the pattern elements.

The dark morph *P. glaucus* utilizes very little yellow pigment in its genetically intended color pattern. Primarily, yellow pigments exist as less than fully saturated “cream-colored” spots on the dorsal wing margins, and as analogous marginal spots accompanied by additional dusting in the Border Gaps on the ventral surface. Aside from the very occasional occurrences of the yellow Forewing Discal Cell Spots (Fig.

13) and the yellowed abdominal tip (Fig. 11), the fully saturated papiliochrome yellow does not occur in dark morph wild individuals. Both the PCE1 and PCE2 experiments demonstrated that to varying degrees, the formation of fully saturated yellow scales *can* be induced by coldshock. However, in comparing some types of PCE1 and PCE2 color aberrations, there is strong evidence to indicate that individuals yellowed by coldshock-induced Color Reversion represent a group that is mutually exclusive from the group of individuals that exhibit blue-based Color Replacement.

One rather surprising similarity between PCE1 and PCE2 is the fact that certain aberrations can occur in multiple colors. So, at least in some cases, the shape and location of the aberration occurred independently of the color synthesized to fill the aberration. This is illustrated in Fig. 14 showing the Pistol Grip Aberration and in Fig. 19 showing the Banded Aberration. These particular aberrations are defined by the space enclosed within their black Pattern Element boundaries. However, the pigment color synthesized to fill these spaces is apparently determined by whether the individual is a yellow-based Color Reversion individual or a blue-based Color Replacement individual.

Yellow Forewing Discal Cell Spot

As previously proposed above, the yellow forewing discal cell spot is probably the result of Pattern Drift which causes Forewing Stripe 2 to converge distally towards Forewing Stripe 3. This creates an encirclement of the scales between the stripes that would normally be contiguous with the remainder of the background scales. These “hard-wired” stripes, although dislocated, are still subject to the earlier fate determination that maintains their ability to be black, and aids their possible ability to inhibit chemical flow across the stripes. This spot likely cannot form without the convergence of the pattern stripes which isolate it from the remainder of the wing. This encirclement must also result in conditions where a surplus of BAS occurs within this spot in order for the scales to synthesize the yellow pigment. It cannot be determined from our experiments whether the yellow scales in the spot are “fate determined” to be yellow, or whether the scales are “fate determined” to be melanic but are forced into the yellow synthesis pathway by the existence of BAS within the spot. It also cannot be determined by our experiments either how or why BAS is produced within the spot. More experimentation of this aberration is needed.

Color Reversion Yellow vs. Color Replacement Blue Aberrations

The blue-based color aberrations generated in PCE2 were not seen in PCE1. As theorized above, blue-based Color Replacement aberrations appear to comprise a category that is predominantly mutually exclusive from yellow-based Color Reversion aberrations. However, these blue-based aberrations include the subcategory of greyish-white aberrations, and the “blueness” or “whiteness” appears to be dependent on

the saturation level of the blue pigments. On several of the blue-based specimens, the Hind Wing Distal Band Spots are silvery-white rather than creamy-yellow. Also, the ventrals of some of the bluest specimens are bluish-brown rather than yellowish-brown. Once the blue tendencies of an individual have been established with coldshock and isolated by selective breeding, an Aberrant Trajectory is initiated that virtually precludes yellow Color Reversion aberrations from occurring. Another indication is that none of the blue-based aberrant specimens displayed any of the body yellowing that was so consistent in the yellowed specimens. The only common exception to this conclusion is the yellow Forewing Discal Cell Spot, which is the result of Pattern Drift in conjunction with innate proclivities to revert to the ancestral yellow.

Lightened Ventral Aberrations

Lightened ventrals occur in the wild to varying degrees, often to the extent of being somewhat disproportionately light. However, our coldshock experiments have demonstrated far more latitude in the location and in the severity of this lightening than in wild individuals. Considering how many millennia the dark morph *P. glaucus* has maintained its ecological and genetic stability, it is surprising how easily and quickly the ventral surface can adapt into and stabilize as a lighter version of its melanic self in subsequent generations. PCE2 has shown that various zones of the ventral wing appear to be independently capable of disproportionate lightening. Further experimentation may reveal why these zones act somewhat independently. In some cases the forewing M_3 vein functions as a boundary between differentially lightened adjacent zones. Further study is warranted to determine the importance of this observation.

In PCE1 we demonstrated that coldshock can cause ventral lightening/yellowing to occur as a consequence of Color Reversion from the melanic brown to the papiliochrome yellow. Sometimes this ventral lightening occurred in proportion to the degree of dorsal yellowing, and sometimes it occurred independently when no dorsal yellowing was present. In PCE2, we produced a few F1 specimens with yellowed ventral lightening similar to the PCE1 specimens. The yellowed-ventral Color Reversion individuals in PCE2 were universally accompanied by yellowed dorsal surfaces, usually within the range of the Group 1 and Group 2 aberrations that we produced in PCE1 (specimens 2 and 3 in Fig. 12). None of these yellowed PCE2 individuals were bred further, so although we believe that these individuals would have initiated Aberrant Trajectories that would have produced increasingly yellow specimens similar to those produced in PCE1, we were unable to test this presumption.

By contrast, the majority of the PCE2 light ventral specimens exhibited a color range that was distinctly greyish-white, which presumably occurred as Color Replacement rather than Color Reversion. The PCE2 specimens that exhibited greyish-white ventrals were universally associated with dorsals that did not exhibit dorsal yellowing. In the most severely affected

individuals, the greyish-white ventrals were accompanied by intensified blue dorsals, which leads to the conclusion that the greyish-white coloration is at least partially linked to the dorsal blue coloration. Further, when present, dorsal and ventral linked coloration shifts appear to be mutually exclusive, being expressed by either Color Reversion when yellow or Color Replacement when blue or greyish-white.

Insufficient Scale Quantity (ISQ) Aberrations

ISQ aberrations are a phenomenon unto themselves. They are not color based, and appear not to be limited to particular colors. ISQ aberrations are often combined with color aberrations to yield a wide range of variation in the affected individuals. In PCE2 these aberrations appeared in all the generations despite the fact that we never bred any affected individuals. Dark morph females were affected more than males by a significantly higher frequency, probably at least by a factor of four. In a more limited previous experiment we conducted on yellow morph females, no ISQ aberrations were produced. We have not experimented enough with ISQ aberrations to form a conclusion, but it appears likely that the dark morph is more susceptible to ISQ aberrations.

A Final Question and Commentary about Inconsistent Coldshock Results

Why are our "results" not more consistent? In other words, *Why don't all of the specimens deviate to the same extent?* We have pondered this question at length, both in an effort to understand the critical particulars of our experiments, and also to increase the percentage yield of radical aberrants.

First, the degree of aberration has a genetic component in terms of, *What is the particular genome capable of deviating to?* For example, if the genetics of an individual do not produce much orange, then the resulting aberrant specimens will not accentuate the orange. Similarly, if the individual genetics tend towards blue, the extent of the blue in the descendents can be accentuated through coldshock. The genetic tendency of an individual can be intensified or diminished by coldshock, *a response to environmental conditions*. Then, the environmentally created coldshock aberration can apparently be further intensified or diminished through selective inbreeding, thereby becoming a somewhat *heritable genetic result*.

Second, the "controllable" factors of temperature and timing are actually NOT very controllable on an individual by individual basis. *What is meant by this?* Every larva is developing on its own individual timeline. This is evidenced by the fact that "fast growers" develop from emergent larva to adult in about five weeks, and "slow growers" from the same broods take over two months for the same development. Therefore, it is obvious that the "rate of development" for each individual is different. Given the individual differences in the developmental timeline, even with precise timing of the chill phase and the precise final temperature, it is impossible

to know if the initiation of the coldshock procedure is occurring at the same point in the developmental timeline for each individual larva in the brood. Further, we do not know if the developmental timeline is linear or nonlinear, i.e., *are some stages of development accelerated or decelerated on an individual basis?* Because of these variables, it can be concluded that it is impossible to predict which individuals will be affected most severely or in what manner. Therefore, a particular aberration cannot be produced consistently even when the initiation of the coldshock procedure is very precise in its application. It is likely that a higher percentage of individuals have the ability to become severely aberrant when the coldshock technique is precisely applied at the exact critical window of opportunity. However, given the inconsistent developmental timeline of the individuals, it is impossible to know when the critical window is occurring.

As a result, it can be concluded that it is impossible to produce an entire coldshocked brood with the same type and degree of aberration, despite knowing most of the variables and adjusting our technique accordingly to accommodate for differences in individual developmental timelines. We ultimately obtained a 10% to 20% ratio of individuals with moderate to severe coldshock-induced aberrations using our coldshock procedure. This percentage rate for moderate to severe aberrants is probably typical for most coldshock experiments.

In addition, it appears that certain particular aberrant characteristics are easier to induce and are consequently more common. In particular, we have observed and produced the yellow Forewing Discal Cell Spot in numerous distinct populations of *P. glaucus*. Indeed, experiments on the PCE1 Alto Pass population descendants resulted in approximately 70% of the adults manifesting some degree of this aberration. Our Elgin population in PCE2 showed this aberration less often and less severely, but still 20% to 30% in most of our generations. And most interestingly, the severity of this yellow discal spot aberration is *independent* of the severity or absence of the background yellow dusting.

So the answer to the question of why our results were not more consistent, may be a function of inconsistent developmental timelines, and not necessarily the result of incorrect application of the coldshock procedure, or even the result of individual genetic tendencies.

Summary of Results from PCE1

Through the use of the coldshock technique described in this paper, it has been demonstrated that:

1. Genetically dark morph females of *P. glaucus* can be forced to produce yellow scales in the background areas of the wings and body where only melanic scales should exist.
2. The intensity of the yellowing effect is variable from minimal to almost completely yellowed over the course of a

few generations, in a complete and uninterrupted cline that we have referred to as an Aberrant Trajectory.

3. The yellowing effect does not materially affect the underlying stripe or border pattern in any way.
4. The yellowing effect does not materially affect the red, orange, cream-colored, or blue scale placement or distribution.
5. The yellowing effect is potentially heritable when used in conjunction with selective inbreeding, as non-coldshocked diapausing descendants of coldshocked parents exhibit a very wide range of yellowing without themselves having been subjected to coldshock.
6. The monomorphic *P. glaucus* males are virtually unaffected by this coldshock technique, exhibiting only minor alterations to the stripes and borders, mostly traceable to Pattern Drift and Pattern Breakdown.
7. Identical chill-phase conditions and timing do not guarantee identical aberrant results. An individual dark morph *P. glaucus* is capable of displaying various abnormal intermediate morphs, partially depending upon when in its individual developmental timeline the coldshock is applied. It has thus far been impossible to predict which pattern elements will change, and to what degree they will transform.
8. Four consistent "rules" appear to govern the yellowing process, including: 1. The yellowing is more pronounced distally, 2. The basal zone is significantly darker, 3. The dorsal and ventral surfaces undergo relatively proportional yellowing, with the ventral being lighter, 4. The bodies appear to be pigmented independently from the wings.

Summary of Results From PCE2

1. The range of possible coldshock aberrations in *P. glaucus* dark morph females was expanded to include not only the wing-wide yellowing caused by Color Reversion (PCE1), but also abnormal color patterns caused by Color Replacement, disproportionately lightened ventral aberrations, and the Insufficient Scale Quantity (ISQ) aberrations resulting from improper scale formation.
2. The monomorphic *P. glaucus* males were not affected by coldshock-induced Color Replacement, and were again virtually unaffected by this coldshock technique, exhibiting only occasional alterations to the stripes and borders resulting from Pattern Drift, Pattern Breakdown, and ISQ aberrations in a few instances.
3. Color Reversion and Color Replacement are the two primary types of wing color aberrations occurring in coldshocked dark morph *P. glaucus* females. Identical aberrations can occur in different colors. Many of the aberration types are defined by the space enclosed within their black Pattern Element boundaries, while the pigment color

synthesized to fill these spaces is determined either by Color Reversion or Color Replacement proclivities.

4. Yellow-based Color Reversion individuals and blue-based Color Replacement individuals appear to be the result of mutually exclusive Aberrant Trajectories that are unlikely to result in specimens that incorporate elements of both trajectories in combination.

5. In addition to the Black stripe and border Pattern Elements, mid-cell lines were detected that also influence the color distribution and pattern stability.

6. PCE2 resulted in a substantially different assortment of types of aberrations than those occurring in PCE1, possibly giving support to the theory that different populations of *P. glaucus* have evolved differing coldshock aberrant capabilities.

7. Ventral lightening can be wing-wide or zonal, with the general tendencies being that the hind wings are usually more affected than the forewings, and the lightening is usually more pronounced distally. In addition, the forewing M_3 vein appears to be influential in certain wing lightening aberrations.

8. ISQ aberrations appear to be much more frequent in dark morph females than in yellow morph females or monomorphic males. When the wings are differentially affected, three general rules apply. First, the dorsal surface is usually affected more than the ventral surface. Second, the forewings are usually more affected than the hind wings. And third, the ISQ aberration is bilaterally symmetrical.

9. Again in PCE2 it was demonstrated that most if not all of the coldshock aberrations can become heritable via selective inbreeding, and can be intensified over successive generations. Eventually these aberrations become somewhat stable, yielding similar aberrations in their descendents without further application of coldshock.

10. Identical chill-phase conditions and timing do not guarantee identical aberrant results. An individual dark morph *P. glaucus* is capable of developing various aberrations depending upon when in its individual developmental timeline the coldshock is applied. Presently, it is impossible to *predict* which color pigments will change, and to what degree they will transform. However, Aberrant Clustering was noted in several generations. This indicates that although the *prediction* of the nature and extent of particular aberrations in individual specimens is impossible due to individual developmental timeline differences, it *is* possible to assume that if there were some way to exactly synchronize groups of individuals, many of their resulting aberrant characteristics could be identical.

11. Even the most extreme of the blue-based aberrant individuals did not display body yellowing, another indication that the yellow and the blue Aberrant Trajectories are mutually exclusive.

12. Forewing Discal Cell Spots are always yellow, being the result of Pattern Drift enclosing the spot and Color Reversion causing the yellow color to occur.

13. Approximately 25 different types of aberrant wing patterns were identified in our experiments. Factoring in various color possibilities and various combinations of aberrations, it is possible to generate hundreds of unique aberrant wing patterns.

CONCLUSION

Coldshock affects different species in different ways, or for that matter, different populations of the same species in different ways. The publication of this paper represents only the beginning of a continuing exploration into how coldshock affects *P. glaucus*. The experiments summarized in this paper have greatly expanded the known universe of possible coldshock aberrations in *P. glaucus*. Coldshock in conjunction with selective inbreeding has allowed us to create an exceptionally wide range of aberrations, all of which originated from the same limited gene pools.

The coldshocked specimens photographed and described in this paper are not only visually remarkable, they also represent the amazing ability of a butterfly species to contend with, adapt to, and triumph over some very extreme physical conditions. The fact that many of the specific aberrations described here have been shown to be somewhat heritable may provide insight as to how and why different species mutate, stabilize, and become localized into regional forms. Also presented here were various examples of Aberrant Clustering, a strong indication that synchronous individuals with sibling DNA can exhibit identical aberrations as they develop along identical timelines. These findings may aid future researchers in determining which specific genes allow certain types of aberrations to occur, how wing scale development alters to synthesize unintended pigments, and even how chemical signaling in the developing chrysalides progresses across the wing membrane. Multiple avenues of study exist that will find this research relevant.

It has been attempted here to offer insights into and explanations for the observed visual effects induced in these specimens. More importantly, it has also been attempted here to relate the results of our experiments to previously published authors' works in an effort to create a more unified understanding of the coldshock phenomenon and how coldshock can be used as an effective tool in unraveling the mysteries of butterfly development. Our experimentation with coldshock is ongoing, with our intention being to expand our range of understanding and explore every different type of aberration that can be environmentally produced in *P. glaucus*.

PME1 (Perlman Mosaic Experiment 1), the second paper in this series, is part of this ongoing experimentation. In PME1 the descendents of the PCE2 specimens started spontaneously producing mosaic offspring, which we subsequently bred.

We have reared these mosaic specimens for over thirteen generations with some totally unexpected, and in some cases spectacular results. We believe all of our experiments have opened the door to many new exciting areas of research, and we hope that our presentation of these experiments will stimulate the interest of other researchers in pursuing further investigation in this regard.

We would sincerely welcome any comments or input from other researchers regarding our experiments.

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SUPPLEMENTARY MATERIALS

P. glaucus Wing Pattern and Venation Maps

For the purposes of creating this Wing Pattern Map, we have used a yellow morph female *P. glaucus* as the example because the pattern is more visible. However, all of the identical black pattern elements are present in the dark morph female underlying the melanic background, in the monomorphic yellow male, and on the ventral surface of both as well. The Wing Venation Map uses the Comstock-Needham nomenclature. The specific wing location references of several

of our observed coldshock aberrations are difficult to discuss using the photographed specimens alone, but are immediately identifiable when compared to the Wing Pattern Map. The names of the pattern elements used in this paper are our own and are referred to differently in other literature. This was done for our own ease of reference, and to more descriptively illustrate their relevance specifically to *P. glaucus*. The labeled details are kept to a minimum in order to facilitate easier use of the Wing Pattern Map.

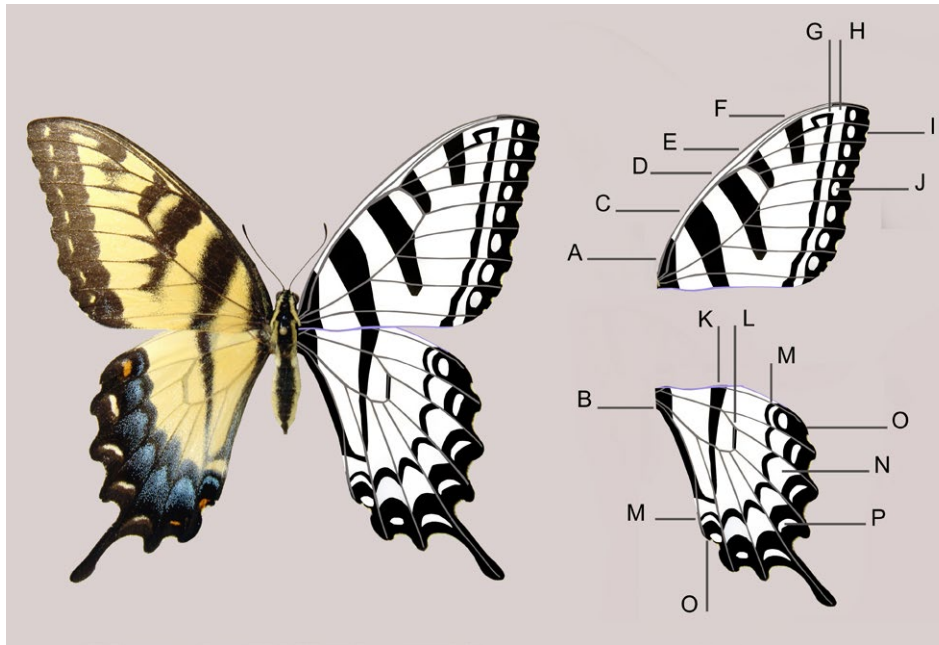


Fig. 33 *P. glaucus* Pattern Map

- A – Forewing Basal Border
- B – Hind Wing Basal Border
- C – Forewing Stripe 1
- D – Forewing Stripe 2
- E – Forewing Stripe 3
- F – Forewing Stripe 4
- G – Forewing Border Proximal Band
- H – Forewing Border Gap
- I – Forewing Border Distal Band
- J – Forewing Border Distal Band Spots
- K – Hind Wing Stripe 1
- L – Hind Wing Stripe 2
- M – Hind Wing Border Proximal Band
- N – Hind Wing Border Gap
- O – Hind Wing Border Distal Band
- P – Hind Wing Border Distal Band Spots

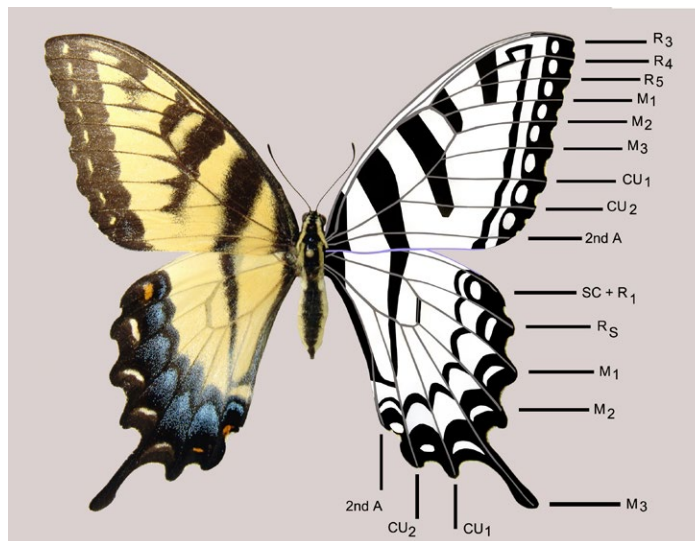


Fig. 34 Wing Venation Map Showing The Veins That Extend To The Wing Outer Margins (Comstock-Needham Nomenclature)

Summary of Literature Cited

In an effort to facilitate the usefulness of this paper, we have included this section summarizing an overview of selected previous works by other authors. Some of the summaries of the research cited here contain simplified background explanations pertaining to Lepidoptera wing color expression generally, and some are specific only to *P. glaucus*. These abbreviated explanations are aimed at readers who are less familiar with these subjects and they are not intended to take the place of the descriptions found in the original literature. They are provided primarily for context only, and generally our intention is to relate them strictly to *P. glaucus*. In many ways, our coldshock experimental work has relevance to each of these previous avenues of research. Many of our assumptions and opinions about our *P. glaucus* coldshock aberrations are based on our interpretation of the relevance of the conclusions drawn by the following authors as the result of their experiments. It is hoped that the presentation and discussion of our coldshock-induced aberrations will be more understandable when considered and evaluated within the context of these previous authors' works.



Fig. 35 Comparison Of Pattern Map To Normal Male And Female Specimens

The sample specimens in Figure 35 are typical of wild collected males and females from Elgin, Illinois, U.S.A. Notice that the dorsal and ventral patterns are essentially analogous, in that all of the black pattern borders and stripes are present on BOTH surfaces. In addition, most of the black pattern elements are analogous on the forewing and hind wing. Differences in shading, color, and intensity do not overrule the pattern element stability. The Hind Wing and Forewing Border Gaps are more obvious on ventral surfaces where they are populated by colored scales rather than by melanic scales. Many of the coldshocked individuals produced in our experiments manifest their aberrations within the constraints imposed by these pattern elements.

1. In 1980, H. Frederik Nijhout published a paper based on experiments conducted on the Nymphalid butterfly *Precis coenia* (Hübner, 1822), (Nijhout, H. F. 1980a). He demonstrated that that butterfly wing pigments are laid down in an ordered sequence, and that certain elements, like the red discal bars, initiate scale pigment determination and scale development prior to those scales that comprise the background wing colors.
2. In 1984, H. Frederik Nijhout published a paper based on the coldshock experiments he conducted on three species of Nymphalid butterflies (Nijhout, H. F. 1984). He listed seven “striking features” about the resulting coldshock aberrant specimens. The three most relevant to our experiment were that individuals of a “synchronous cohort” were affected to differing degrees of severity, the aberrant specimens induced by coldshock resulted in a continuous cline of intensities which he dubbed an “Aberrant Gradient”, and the various aberrants were symmetrical right to left. **The results of our *P. glaucus* experiment demonstrated all three of these characteristics.** Of additional interest, although the coldshock technique used in our experiment incorporated a similar timeline to Nijhout’s, a slightly higher temperature was used in our experiment that never dropped below freezing.
3. In 1985, H. Frederik Nijhout published a paper detailing cautery eyespot formation responses in the Nymphalid *Precis*

coenia (Hübner, 1822), noting differences in the severity of the effect (Nijhout, H. F. 1985a). He presented two options as to the cause of his observations. Either, wing surface “development is asynchronous so that at any one time they are not in equivalent stages of colour pattern determination”, or “the various wing surfaces may simply differ in their threshold to the “cautery-response”. **His experiment may point to a possible contributing cause as to why our coldshocked *P. glaucus* specimens were yellower distally than proximally.**

4. In 1986, David B. Ritland published a paper on the effects of rearing *P. glaucus* at higher than normal temperatures (Ritland, David B. 1986/87). The adults that eclosed displayed wing-wide yellowing in which genetically dark morph females exhibited varying degrees of yellow background scales replacing the normal melanized scales, revealing the pattern stripes, and indicating some type of shift in the pigmentation pathways. He sorted the resulting specimens into 5 groups that constituted a cline of intermediate morphs corresponding in our classification system to “Unaffected”, Group 1, Group 2, Group 3 and Group 5. **Based on comparison to his photographs, the cline resulting from our experiment manifests a similar type of aberration.** It is especially interesting to note that both coldshock and “heat shock” can interrupt the pigment determination process with very similar results, even if the exact causes turn out not to be the same.
5. Beginning in 1998, P. Bernhardt Koch et. al. commenced

a series of experiments in which they determined that pigment synthesis in the background coloration in *P. glaucus* proceeds in an ordered sequence in which the yellow papiliochrome precedes the melanin (Koch, P. B., et. al. 2000a & 2000b). Next, they deciphered and identified the chemical basis and a model for pigment synthesis in *P. glaucus*, including the underlying fact that the yellow papiliochrome and the black melanin share common chemical precursors that are regulated both chemically and temporally (Koch, P. B., Keys, D. N., Rocheleau, T. et al. 1998). Possibly most important and relevant to our experiments, they demonstrated that wing scales which are destined to become yellow, mature earlier than scales that are destined to be melanized, and that both the black pattern elements and the brown background scales are pigmented contemporaneously (Koch, P. B., Lorenz, U., Brakefield, P. M. & French-Constant, R. H. 2000b). ***Accepting that yellow synthesis precedes melanin synthesis, our results indicate that coldshock may accelerate the developmental pathway for these scales, which presumably causes the genetically predestined melanic scales to mature early and synthesize yellow instead.*** Also demonstrated was evidence that yellow papiliochrome pigmented scales do *not* become repigmented by melanin later in development, but rather that color choice in these scales is restricted to an *either/or* possibility of color selection (Koch, P. B., et al. 1998). ***This may partially explain why some of our specimens exhibited a distinct “clumpiness” in the distribution of yellow vs. melanic scales.***

6. In 2008, Jogi M. Otaki presented the results of tungstate injection experiments in the Nymphalid species, *J. orithya* (Linnaeus, 1758), in which tungstate application produced similar results to coldshock-induced effects (Otaki, J.M. 2008). He then proposed that, “the color-pattern formation process is conceptually divided into four distinct sequential steps: signaling, reception, interpretation, and expression”. ***The 4-step process is referred to several times in the analysis section of this paper.*** (Note: The Otaki model is a sequentially-based model, not a temporally-based model. What we mean by this is that his model indicates which things need to occur and in what order they occur. His model does not tell us how many hours are needed to complete a process, how these processes can be affected by random severe alterations in the actual developmental timeline, if various processes are simultaneous, or if the resulting distortions in these processes are dependent on their particular location on the developing wing.)

7. In 2010, Jogi M. Otaki et. al. published a paper documenting the results of experiments on the Lycaenid species *Zizeeria maha* (Kollar, 1844), investigating a pattern aberration that occurs in the extreme northern end of its geographic distribution range (Otaki, et. al. 2010). This pattern aberration was induced in the laboratory by coldshocking individuals from unaffected southern populations, and coldshock was subsequently found to increase the percentage of affected individuals in succeeding generations. Continued breeding resulted in finding the aberration to be heritable in

all succeeding generations even without coldshock, and even when these generations were paired with unaffected wild-caught individuals. They found that the aberrations were more prevalent in females, that different populations react differently to coldshock, and that inbreeding coldshocked individuals causes a severe drop off in the fertility viability of the strain. ***These are all observations that were demonstrated to exist in our coldshocked P. glaucus.***

Terms Used in this Paper

There are five concepts that are introduced in this paper. Because they have been used as terminology throughout this paper, we have expanded and further defined them here. These five concepts are based on personal observations during our many years of breeding projects. The terms are Pattern Drift, Pattern Breakdown, Aberrant Bracketing, Aberrant Trajectory, and Aberrant Clustering. We have observed these phenomena in several other species we have bred, but they are particularly important when analyzing *P. glaucus* aberrations.

Pattern Drift:

We use the term Pattern Drift to describe minor dislocations and migrations of the various black pattern elements to slightly displaced positions on the wing surface. Pattern Drift is most frequently seen in the forewing stripes. Often the forewing stripes look “windblown” as if the relatively distinct stripe edges have been pushed, usually distally, especially more pronounced adjacent to the wing veins. This gives the stripes a distinctly scalloped appearance. This stripe scalloping is very common and even typical in wild-collected individuals. However, many of our coldshocked specimens exhibited this effect more severely. We would also include severe thickening or narrowing of the pattern elements in the category of Pattern Drift.

Pattern Breakdown:

We use the term Pattern Breakdown to describe a generalized or localized deterioration of the pattern borders and stripes, in most cases creating a fuzzy, faded looking, or blurred effect in the otherwise hard-edged pattern elements. In other instances, especially in the dorsal borders, there sometimes appears a dusting of yellow or blue scales in the otherwise very solid black areas.

Author’s Note: Pattern Drift and Pattern Breakdown can occur in the same specimen, and very often both aberrations do occur in the same specimen. However, they each occur alone in sufficient quantities of specimens to warrant treating them as two separate types of aberrations.

Aberrant Bracketing:

We use the term Aberrant Bracketing to describe a phenomenon we have observed consistently when breeding aberrant specimens. When one of the parents of a brood is

mildly to moderately aberrant, many of its same-sex offspring will exhibit the same type of aberration to varying degrees, with the original aberrant parent falling somewhere in the middle of the cline of resulting aberrant offspring. Of most importance, an aberrant parent will likely produce some offspring that are more severely aberrant than itself. This phenomenon creates an ever-increasing maximum severity level, and provides a mechanism for continued severity increases in succeeding generations. Aberrant Bracketing was used as our primary tool in intensifying the aberrations we wished to explore further.

Aberrant Trajectory:

We use the term Aberrant Trajectory to describe a phenomenon in which a particular type of aberration will continue to intensify through subsequent generations, along a moderately predictable cline of severity, always remaining within a limited range of possibilities. For example, if an aberrant specimen is abnormally blue, its offspring will likely become more blue rather than suddenly shifting to yellow. Aberrant Trajectories require an individual to possess a suppressed genetic capability, but that genetic capability does not become expressed without the proper external forces (like coldshock). Aberrant Trajectories are not initially the most useful tool for predicting future aberrants, but they are a fairly useful tool for looking backwards to trace the origins of certain aberrations. Once these aberrant origins are known, it can be predicted that other individuals with similar baseline aberrations will continue along the known Aberrant Trajectory in subsequent generations. As an example, in

Fig. 12, individuals 2 through 7 constitute a five-generation Aberrant Trajectory. We have not demonstrated that a mother like specimen 2 can directly produce a daughter similar to specimen 7. However, it seems highly likely that by starting with an initial mother like specimen 2, an Aberrant Trajectory can be initiated that will culminate with individuals like specimen 7 after several generations. We believe that in this scenario, the concept of an Aberrant Trajectory can be used to predict similar results in future breeding experiments. Finally, an Aberrant Trajectory is different from a cline in that the term cline is often used to describe the range of intensities within a single generation, while an Aberrant Trajectory must be multi-generational and intensifies over the course of successive generations.

Aberrant Clustering:

We use the term Aberrant Clustering to describe a phenomenon in which a particular type of aberration occurs in multiple sibling same-sex individuals that eclose within a very close time frame, and that aberration usually does not occur again within the same brood. We have observed this phenomenon frequently in numerous species. As related to coldshock, most often Aberrant Clustering occurs to two or more individuals that eclose within hours of each other. While this is a totally unpredictable phenomenon, it is a probable indication that these sibling individuals were progressing on the identical developmental timeline, and therefore experienced the identical environmentally stressful conditions within the identical window of opportunity.

Additional Examples of Wing-Wide Yellowing - PCE1 Group 1 to Group 6



Fig. 36 PCE1 Group 1 Yellowed Dark Morph Females

This composite photo shows examples of Group 1 yellowed coldshock aberrations, defined as having 5% to 20% yellow scales replacing the normal melanic scales. This is a very minor manifestation that could be easily overlooked if encountered in the wild. However, even though these specimens are only minimally yellowed, they indicate the proclivity to yield a lineage that can probably be coldshocked to produce more severely yellowed offspring in subsequent generations. The yellow Forewing Discal Cell Spots are also commonly seen in coldshocked individuals, but result from the unrelated cause of Pattern Drift, presumably caused by the same convergence of the stripes that was observed in the male specimens. Group 1 individuals were produced in all PCE1 generations.



Fig. 37 PCE1 Group 2 Yellowed Dark Morph Females

This composite photo shows examples of Group 2 yellowed coldshock aberrations, defined as having 21% to 35% yellow scales replacing the normal melanic scales. Group 2 individuals look “unusual” by comparison to normal individuals. They are “minor” aberrants, and recognizable as such. This grouping displays significant ventral lightening in the form of yellow scales mixed with poorly saturated melanic scales. Yellowing of both the dorsal and ventral surfaces is more pronounced distally. Notice also that the yellowing occurs on the dorsal hind wing within the area where usually only blue dusting occurs. The yellow scales have replaced the melanic scales, with the blue scales being pigmented as genetically intended. The yellow Forewing Discal Cell Spot is prominent in the above four specimens, which is more of a coincidence than a trait linked to the coldshock yellowing. Group 2 individuals were produced in all PCE1 generations.



Fig. 38 PCE1 Group 3 Yellowed Dark Morph Females

This composite photo shows examples of Group 3 yellowed coldshock aberrations, defined as having 36% to 50% yellow scales replacing the normal melanic scales. The dorsal yellowing clearly reveals the underlying black “tiger” stripes, with the basal zone being relatively unaffected. This dark basal zone is a typical characteristic of coldshock-induced yellowing. Additionally, the bodies also become yellow as the severity of the wing yellowing occurs. Interestingly, the yellowing of the bodies seems to occur despite the fact that the adjacent basal wing sections remained darker. The two specimens at right could be mistaken for severely melanic yellow morph females if their origins were unknown. Group 3 individuals were produced in all PCE1 generations.



Fig. 39 PCE1 Group 4 Yellowed Dark Morph Females

This composite photo shows examples of Group 4 yellowed coldshock aberrations, defined as having 51% to 65% yellow scales replacing the normal melanic scales. The specimens in this sampling first appeared in the F2 generation and are the coldshocked offspring of Group 2 mothers. The basal zone still remains populated by mostly melanic scales, which is an indication of the stability of the dark morph. The bodies are almost fully yellow, with only the black dorsal stripe being unaffected. Group 4 specimens give the impression that they are severely melanic aberrations of the yellow morph, rather than severely yellowed aberrations of the dark morph. It is also noticeable in these specimens that the yellow vs. melanic scales tend to look somewhat “clumpy” in their distribution rather than being uniformly distributed.



Fig. 40 PCE1 Group 5 Yellowed Dark Morph Females

This composite photo shows examples of Group 5 yellowed coldshock aberrations, defined as having 66% to 80% yellow scales replacing the normal melanic scales. These specimens first appeared in the F2 generation and are the offspring of Group 2 and Group 3 mothers. The wings being mostly yellow now give the impression that these are yellow morph *P. glaucus* that have been moderately dusted with melanic scales, rather than being dark morph *P. glaucus* that are severely dusted with yellow. The basal darkening is still largely unaffected, but it is now more obvious that Forewing Stripe 1 and Hind Wing Stripe 1 create some type of “barrier” that helps to maintain the genetically programmed melanic color, despite the fact that the bodies are now almost completely yellowed. The yellow Forewing Discal Cell Spot is still evident, and can be more easily traced to the outward Pattern Drift of Forewing Stripe 2.



Fig. 41 PCE1 Group 6 Yellowed Dark Morph Females

This composite photo shows examples of Group 6 yellowed coldshock aberrations, defined as having 81% to 95% yellow scales replacing the normal melanic scales. The Group 6 specimens pictured above are from the F4 and F5 generation and are the offspring of Group 3 mothers. All of the bodies are yellow including the legs, with no trace of the genetically programmed melanic color except for the black body pattern stripes. The wings being almost totally yellow now give the impression that these are yellow morph *P. glaucus* that have been very minimally dusted with melanic scales. The basal darkening is now very significantly diminished, with Forewing Stripe 1 and Hind Wing Stripe 1 still creating a buffering barrier that helps to maintain the genetically programmed melanic color. The F5 specimen on the right is the most extreme of the Group 6 females, with absolutely no remnant of the genetically programmed melanic scales on its ventral surface, and is thus indistinguishable from a yellow morph female *P. glaucus* on its ventral.

One-of-a-Kind Specimens from PCE1

As a final tantalizing glimpse into the potential of coldshock, we have decided to include Fig. 42. These four specimens represent extreme examples of the coldshock effect that fell outside of the parameters of our intentionally bred aberrations. The two specimens on the left are more representative of the specimens we bred in PCE2. We believe that these aberrations could have been intensified in subsequent generations using our coldshock technique if we had not been concentrating our efforts instead on the yellowed specimens. The two specimens on the right are extremely yellowed, while also exhibiting much more blue than the other PCE1 specimens.

One-of-a-Kind Specimens From PCE2

PCE2 yielded some extreme specimens that were one-of-a-kind aberrations. These are unique enough that they didn't fall into any of the previously discussed categories. Some of these specimens fall outside of the strictest parameters of the PCE2 experiment. The specimens in Fig. 43 were the result of either multiple chill-cycles, or were from subsequent generations after we stopped administering the coldshock. The multiple chill-cycle specimens may have sustained as many as three separate chill phases. In all cases however, cold temperatures were responsible for the aberrations. There were many more specimens that could have been included in this section, but the four photographed specimens are the most varied and interesting.



Fig. 42 One-Of-A-Kind Female Specimens From PCE1

This composite photo illustrates four of the most outstanding specimens produced by this series of experiments, the first two of which do not fall into the Color Reversion category. The first specimen is an extremely dark specimen with diminished markings and significantly reduced blue, also showing no evidence of the outermost forewing tiger stripe. The second specimen displays an incredibly expanded strongly blue zone on the dorsal hind wing that is correspondingly lightened on the ventral hind wing, also showing a virtual loss of outermost forewing tiger stripe. The third specimen is a spectacular specimen displaying a sharp demarcation between the dark basal zone and the highly yellowed mid and outer wing, and also displaying a greater than normal amount of blue on the dorsal hind wing. The fourth specimen is in our opinion, the most spectacular specimen we produced, showing an almost complete Color Reversion except for the still strongly melanized basal zone.



Fig. 43 One-Of-A-Kind Female Specimens From PCE2

This composite shows four remarkable one-of-a-kind individuals, all vastly different from each other. Specimen 1 is an extreme blue aberration with blue in every cell of the Forewing Border Gap, extreme blue on the hind wings, and blue dusting on the forewings extending up past the M_3 vein. Notice also the almost complete absence of the normally prominent uppermost orange Hind Wing Distal Band Spot. Specimen 2 has no blue on the dorsal and virtually no blue on the ventral. It is slightly shaded in its melanic scales accompanied by a minimal ISQ aberration. Specimen 3 is intensely marked with virtually no hind wing blue proximal to the Hind Wing Border Proximal Band, and lots of orange on the hind wing ventral. Specimen 4 was the only such specimen produced. Its markings are intense and the blue trails up the Forewing Border Gap. The blue on the hind wing dorsal is extremely intense and well defined, with a distinctly yellowish curving band inside the Hind Wing Border Proximal Band. The ventral forewings have developed a secondary yellow band in the center of the discal cell. The hind wing ventral is excessively orange with a distinct Banded Aberration, and the blue in the Hind Wing Border Gap is extremely vibrant.